Estradiol With or Without Progesterone and Ambulatory Blood Pressure in Postmenopausal Women

Ellen W. Seely, Brian W. Walsh, Marie D. Gerhard, Gordon H. Williams

Abstract—The purpose of this study was to determine whether transdermal estradiol and intravaginal progesterone given in doses to mimic the premenopausal state would lower blood pressure (BP) in postmenopausal women. Fifteen healthy postmenopausal women were studied in each of 3 conditions: on placebo, after 8 weeks of transdermal estradiol 0.2 mg twice per week, and again 2 weeks after addition of intravaginal progesterone 300 mg/d. Women were studied at each point after 2 days of 100 mmol/d sodium intake. Twenty-four–hour ambulatory BP monitoring was performed, and blood was assayed for estradiol, progesterone, and hormones of the renin-angiotensin-aldosterone system (RAAS). ANOVA with pairwise comparisons was used for analysis. Urinary sodium excretion was similar at each time point. Levels of estrogen and progesterone similar to those in premenopausal women were achieved. On estradiol, nocturnal systolic BP (110±3 mm Hg), diastolic BP (63±2 mm Hg), and mean BP (77±6 mm Hg) fell significantly (P<0.02) compared with placebo systolic BP (116±2 mm Hg), diastolic BP (68±2 mm Hg), and mean BP (82±2 mm Hg). Daytime BP followed the same trend but was significantly lower only for mean BP. There was no activation of the RAAS. The addition of progesterone resulted in no further fall in BP but a significant activation of the RAAS. Thus, contrary to what is often assumed, administration of estradiol with or without progesterone not only did not raise BP but rather substantially lowered BP. This BP-lowering effect may be responsible for the lower incidence of hypertension in premenopausal than in postmenopausal women. (Hypertension. 1999;33:1190-1194.)

Key Words: hypertension ■ women ■ blood pressure monitoring, ambulatory ■ renin-angiotensin system

Premenopausal women have a lower incidence of cardiovascular disease than age-matched men; however, after menopause the incidence of cardiovascular disease in women is similar to that in men. Much of the focus of investigation to explain this change in risk has been on estrogen-induced effects producing a more beneficial lipid profile in the premenopausal woman. However, beneficial changes in lipid profiles appear to account for only 25% to 50% of the cardiovascular benefit seen in premenopausal women, thereby raising the question of other estrogen-induced cardiovascular benefits that may be lost at menopause.

Hypertension is considered a major risk factor for cardiovascular disease. Of note, several studies have shown that the incidence of hypertension is lower in women than men until approximately the age of 51 years. After this time, the incidence of hypertension in women exceeds that in men. Since 51 years is the average age of menopause in the United States, this observation raises the question of whether the loss of the gonadal sex steroids, estradiol and progesterone, contributes to the rise in blood pressure (BP). This possibility is contrary to the common belief in clinical practice that estrogens raise BP. In part, this belief stems from the observed hypertensive effects of oral contraceptives, as documented in the 1970s. However, the effect of standard-dose conjugated equine estrogens, by far the most common form of hormone replacement therapy in the United States, appears to have a neutral effect on BP. This raises the possibility that the effects of hormonal therapy on BP may be both dose and agent specific.

If estrogen and progesterone lower BP, the mechanism is still unclear. Several possibilities have been suggested. Both estrogen and progesterone are vasodilatory. However, when given orally both can lead to activation of the renin-angiotensin-aldosterone system (RAAS). Activation of this system leads to vasoconstriction and volume retention and can lead to an increase in BP.

The present study was designed to study the effects of replacement of the naturally circulating estrogen (estradiol) and natural progesterone given to postmenopausal women in doses to mimic premenopausal levels. To obtain a complete picture of the effect of treatment, 24-hour ambulatory BP and the hormones of the RAAS were assessed after chronic therapy.
Methods

Subjects
Fifteen healthy normotensive postmenopausal women were studied. Normotension was determined by the mean of 3 BP determinations, taken at 5-minute intervals with the subject in the seated position, and was defined as mean systolic and diastolic levels <140/90 mm Hg. All women were at least 1 year from their last menstrual period and had follicle-stimulating hormone level >40 IU before study enrollment. Each had a normal physical examination and mammogram. All had received no hormonal therapy for at least 4 weeks before the study. The study was approved by the Human Subjects Committee of the Brigham and Women’s Hospital. Each participant gave informed written consent, and all procedures were in accordance with institutional guidelines. This study was a substudy of a larger trial determining the effects of treatment with estradiol and progesterone on metabolic factors in postmenopausal women.

Protocol
Nineteen women were enrolled in the trial, and 15 participated in the BP study. The subjects received each of the following 2 regimens in random order according to a double-blind crossover design: (1) transdermal estradiol alone for 8 weeks followed by the addition of intravaginal micronized progesterone suppositories for 2 weeks at weeks 9 to 10 and weeks 13 to 14 and (2) placebo patch and placebo suppositories following the same regimen. Transdermal estradiol was administered as two 0.1-mg patches (Estraderm, Ciba-Geigy) twice a week at night. Intravaginal progesterone was administered as 300 mg micronized progesterone (Upjohn) in a nonliquifying base (Unibase, Warner Chilcott Labs) daily at bedtime. Corresponding placebo patches and suppositories were used. Women were counseled regarding intake of 100 mmol sodium per day for 2 days before the study so that their dietary sodium balance would be similar at each study time point.

Study Procedures
Participants were studied during treatment with estradiol or placebo (week 8) and during treatment with estradiol and progesterone or placebo (week 10). Each study time point included collection of a 24-hour urine sample to determine sodium and creatinine and measurement of BP with the use of a 24-hour ambulatory BP monitor. The BP monitor (SpaceLabs) was programmed to measure BP every 30 minutes from 6 AM to midnight, then every 60 minutes from midnight to 6 AM. Daytime BP was defined as 8 AM to 8 PM and nighttime BP from 8 PM to 8 AM. Systolic, diastolic, and mean arterial pressure and pulse were recorded at each determination. Diaries of sleep and wake periods were kept by the participants during the monitoring. On the day of the 24-hour BP monitoring, BP was also determined with the subjects in the sitting position after 30 minutes of rest with a random zero sphygmomanometer. Three determinations of BP were made at 5-minute intervals and were averaged. Blood for hormone assays was then drawn from an indwelling catheter.

Assays
Blood was analyzed for estradiol, progesterone, and hormones of the RAAS. Estradiol and progesterone levels were determined by radioimmunoassay (Bayer). Blood for total and active renin were measured by immunoradiometric assay (Nichols). Plasma renin activity (PRA) was measured by radioimmunoassay of angiotensin I generation (IncStar). Aldosterone was determined with the Coat-A-Count DPC kit.

Statistical Analysis
Results are expressed as mean±SEM. One-way ANOVA with pairwise comparisons was used to determine differences among the 3 study interventions (placebo, estrogen alone, and estrogen+progesterone).

Results

Demographic Data
Clinical characteristics of the study population are displayed in Table 1. The subjects were primarily white, with an average age of 56±2 years. All were normotensive, and the majority had undergone natural menopause.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of subjects</th>
<th>Age, y</th>
<th>Race, No. white</th>
<th>Weight, kg</th>
<th>Body mass index, kg/m²</th>
<th>Years postmenopausal</th>
<th>No. with natural menopause</th>
<th>Baseline office BP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>15</td>
<td>56±1.5</td>
<td>13</td>
<td>67.3±2.7</td>
<td>24.9±0.9</td>
<td>8.6±1.6</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

Estradiol and Progesterone Levels
As expected, levels of estrogen and progesterone were low during placebo administration. Both during estradiol administration and during estradiol and progesterone administration, the levels of serum estradiol achieved were significantly higher than on placebo (P<0.01) and were similar to each other (52±6 pmol/L [140±16 ng/mL] and 61±7 pmol/L [165±19 ng/mL], respectively). Progesterone levels were significantly elevated only during progesterone administration (3.8±0.3 pmol/L[11.8±1.0 ng/mL]; P<0.01).

Blood Pressure
There were no significant differences in resting morning systolic (placebo, 115±3; estradiol, 111±4; estradiol+progesterone, 108±2 mm Hg) or diastolic (placebo, 73±2; estradiol, 70±3; estradiol+progesterone, 70±2 mm Hg) BPs as determined by random zero sphygmomanometer between interventions. However, with ambulatory monitoring, on estradiol there was a significant fall in nighttime systolic (110±3 mm Hg), diastolic (63±2 mm Hg), and mean arterial (77±2 mm Hg) BPs compared with placebo systolic (116±2 mm Hg), diastolic (68±2 mm Hg), and mean (82±2 mm Hg) BPs (all P<0.02) (Table 2). This effect was also seen in the trend toward lower values during the day, which was significant for mean BP (P<0.02). There was greater variability of BP during the day (8 AM to 8 PM) than during the night (8 PM to 8 AM). When we used 3 mm Hg as a significant change in diastolic BP, 10 of 15 had a fall, 5 of 15 had no change in nighttime diastolic BP, and none had a rise. There was no change in heart rate. The addition of progesterone did not lead to any further change in day or night BP beyond that seen with estradiol alone (Table 2). The number of hours of sleep, as recorded in the diaries during the BP monitoring, did not differ among the 3 study periods. Twenty-four-hour urinary sodium excretion was similar at each study time point (placebo, 100±9; estradiol, 94±8; and estradiol+progesterone, 108±12 mmol).
Effect of Estradiol and Progesterone on Ambulatory BP

### TABLE 2. Ambulatory Blood Pressure Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estradiol</th>
<th>Estradiol and Progesterone</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>122±2</td>
<td>123±2</td>
<td>124±2</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>75±2</td>
<td>75±2</td>
<td>77±2</td>
</tr>
<tr>
<td>Mean BP, mm Hg</td>
<td>89±2*</td>
<td>90±1</td>
<td>92±2</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>80±2</td>
<td>83±2</td>
<td>80±2</td>
</tr>
<tr>
<td>Night</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>110±3*</td>
<td>108±2*</td>
<td>116±2</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>63±2*</td>
<td>61±1*</td>
<td>68±2</td>
</tr>
<tr>
<td>Mean BP, mm Hg</td>
<td>77±2*</td>
<td>75±1*</td>
<td>82±2</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>68±2</td>
<td>70±2</td>
<td>68±2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *P<0.02.

### Hormones of the Renin-Angiotensin-Aldosterone System

Hormone levels on placebo were consistent with those expected on a 100-mmol sodium balance state. With estradiol, there was a significant increase in both total renin (159±20 mU/mL) and prorenin (140±19 mU/mL) compared with placebo (107±20 and 92±11 mU/mL, respectively; both *P<0.05). There was no change in active renin, PRA, or aldosterone levels compared with placebo. When progesterone was added to estradiol, there were significant increases in all components of the RAAS assayed (Table 3). However, as noted above, there was no further change in BP.

#### Discussion

It has been recognized for many years that the premenopausal state is protective against the development of cardiovascular disease. In the past, much of the protective benefit has been attributed to estrogen-induced lipid changes favoring a lowering of LDL and increase in HDL cholesterol. Recently, other cardiovascular effects of estrogen focusing on its potential vasodilatory properties have been examined. The present study demonstrates that postmenopausal hormone replacement therapy is accompanied by a reduction in BP when natural premenopausal hormones are used in a manner that avoids first-pass liver effects and in doses that produce hormone levels similar to those that exist in the premenopausal state.

#### Estradiol and Progesterone

In this study the goal of the estradiol and progesterone treatment was 2-fold: (1) in postmenopausal women, to replace the naturally circulating forms of estrogen and progesterone and (2) to achieve levels seen in premenopausal women. The first goal was achieved by using the transdermal and intravaginal routes of administration of estradiol and progesterone, respectively. For the second goal, the estradiol levels achieved were similar to those of the early to midfollicular phase, and progesterone levels were compatible with those seen in the luteal phase.

#### Blood Pressure

BP was lower during ambulatory monitoring with estradiol treatment. The fall in BP with estradiol was most notable at night, when significant decreases in systolic, diastolic, and mean BP were seen. During the day, only MBP was significantly lower. This appears to reflect the confounding effect of activity on BP, which was more pronounced during the day. Since urinary sodium balance was the same at each study time point, sodium balance could not explain the difference in BP. In addition, the subjects reported no difference in the number of hours of sleep to explain this finding.

Prior studies have demonstrated that a nocturnal fall in BP is a good prognostic factor associated with lesser degrees of future end-organ damage. A study of ambulatory BP in essential hypertensives demonstrated that women (but not men) who demonstrated an absent or blunted nocturnal fall had an excess of cardiovascular mortality in the following 1 to 5 years compared with those who demonstrated a fall. Therefore, the impact of estradiol at night demonstrated in this study may be particularly important in the protective effect of the premenopausal state against both hypertension and other cardiovascular diseases.

There are 3 other published studies of ambulatory BP determination in postmenopausal women receiving estrogen therapy. The present study is the only one to evaluate the effect of premenopausal levels of estradiol and progesterone on BP in postmenopausal women. Akkad et al reported nocturnal lowering of BP in women using chronic transdermal estradiol as opposed to an oral estrogen (Hormonin). The study was not randomized or placebo controlled, and there was no determination of sodium balance. In this study there was an observed lowering of nocturnal BP with transdermal estradiol, in agreement with our present study. However, all women had undergone surgical menopause and therefore were relatively young (mean age, 45.0 years), and progesterone was not administered.

Mercuro et al studied 24-hour ambulatory BP in postmenopausal hypertensive women after the acute administration of estradiol. Although ambulatory BP was recorded, the study was performed in an inpatient setting. The 24-hour BP was lower when women were receiving estradiol than placebo during both daytime and nighttime monitoring. The significant lowering seen in the day is likely due to the limitation on activity level caused by the in-hospital setting but may also represent a different magnitude of effect on hypertensive than normotensive women. Again, the effects of the addition of progesterone were not examined in this population.

A European study with a nonblinded parallel design using 1 mg oral micronized estradiol-17β daily with 5 or 10 mg of progestin also demonstrated a significant nighttime lowering of BP with estradiol treatment.

### TABLE 3. Hormones of the RAAS

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Estradiol</th>
<th>Estradiol and Progesterone</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total renin, mU/mL</td>
<td>159±20*</td>
<td>214±33†</td>
<td>107±20</td>
</tr>
<tr>
<td>Active renin, mU/mL</td>
<td>18.3±2.0</td>
<td>31.5±6.1*</td>
<td>15.1±2.5</td>
</tr>
<tr>
<td>Prorenin, mU/mL</td>
<td>140±19*</td>
<td>182±29*</td>
<td>92±11</td>
</tr>
<tr>
<td>PRA, ng/L · S</td>
<td>0.23±0.04</td>
<td>0.41±0.09*</td>
<td>0.26±0.04</td>
</tr>
<tr>
<td>Aldosterone, pmol/L</td>
<td>302±32</td>
<td>469±72†</td>
<td>250±22</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *P<0.05, †P<0.01 compared with placebo.
mg of the progestin dydrogesterone (days 14-28) showed a lowering of 24-hour ambulatory BP after 12 months of treatment. No difference was seen between the groups receiving the 2 doses of dydrogesterone, but the 24-hour monitoring was performed only during the estradiol administration phase. Therefore it is unclear whether the hypotensive effect would be mitigated by the addition of this progestin.

That no difference could be detected in standard office BP determination in these studies or ours is similar to the BP findings in the larger Postmenopausal Estrogen/Progestin Intervention (PEPI) study. In contrast to the present study, the PEPI study used conjugated equine estrogen, which may have a different effect on BP compared with the naturally occurring estradiol. A difference in BP response to different estrogen preparations was noted by Wren and Routledge, who showed no change in BP after chronic therapy with conjugated equine estrogen (Premarin) but a fall with estrone (Ogen). The failure of finding a cardiovascular benefit to “estrogen replacement therapy” in the recent Heart and Estrogen/Progestin Replacement Study (HERS) may be reflective of the type of estrogen used in the study. The HERS used conjugated equine estrogen and medroxyprogesterone acetate. This estrogen and progestrone combination may not be one that lowers BP.

The Renin-Angiotensin-Aldosterone System

Previous studies using oral estrogens have demonstrated increases in PRA and aldosterone, likely through a hepatic-induced increase in angiotensinogen. Indeed, this activation of the RAAS has been proposed as a potential mechanism of estrogen-associated hypertension. Transdermal estrogen avoids this effect on the liver, and no effect on PRA or aldosterone has been shown in this and prior studies.

In this study we measured both total and active renin and demonstrated that although estradiol increased total renin, it did not increase active renin. An increase in prorenin and total renin was seen without a concomitant increase in active renin or plasma renin activity. This finding supports the observational study by Schunkert et al, which showed that estrogen use is not associated with a rise of active renin. There are several possible explanations for this finding of the present study. It is possible that estradiol accelerates the metabolism of renin. However, this explanation would not account for the increase in total renin seen with estradiol administration. There is an estrogen response element on the promoter region of the angiotensinogen gene and a putative estrogen response element on the renin promoter as well. Thus, one would anticipate that estradiol would increase transcription of renin message, as reflected in the increase in total renin. As demonstrated by this study, however, it is likely that estradiol has another heretofore unknown effect on the RAAS: inhibition of the conversion of precursor renin into active renin. This inhibitory effect is supported in 4 ways: no change in active renin, PRA, and aldosterone levels and a rise in prorenin that parallels the rise in total renin. Angiotensinogen levels were not measured in this study. However, previous studies using transdermal patch estradiol did not report a change in angiotensinogen levels. Finally, this finding is unlikely to be secondary to some peculiarity of the RAAS in the population studied since the addition of progesterone produced a substantial activation of the RAAS. An inhibition of the activation of the RAAS by estradiol may play an important role in its ability to lower BP.

In conclusion, administration of transdermal estradiol with or without progesterone has a hypotensive effect, particularly at night, in postmenopausal women. Furthermore, estradiol appears to have a dual effect on renin: it increases its production but inhibits its activation. Further studies are required to affirm the first conclusion, using a clinical trial format, and to determine the mechanism(s) responsible for the second effect.

Acknowledgments

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References

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