The Effects of Transdermal Estradiol on the Response to Mental Stress in Postmenopausal Women: A Randomized Trial

Graziano Ceresini, MD, Marilena Freddi, MD, Simonetta Morganti, MD, Isabella Rebecchi, MD, Alberto Bacchi Modena, MD, Maurizio Rinaldi, MD, Carlo Manca, MD, Alberto Amaducci, MD, Graziano Del Rio, MD, Giorgio Valenti, MD

PURPOSE: Estrogens inhibit adrenomedullary catecholamine release and catecholamine-mediated responses to stress. We examined whether estrogen supplementation reduces the sympathoadrenal response to mental stress in postmenopausal women.

MATERIALS AND METHODS: We compared the effects of 3-week treatment with transdermal 17-beta-estradiol and placebo in 10 postmenopausal women using a randomized, blinded, crossover design. We measured plasma catecholamine levels and the cardiovascular and metabolic responses to a 15-minute stress with mental arithmetic. Treatments were compared using repeated measures analysis of variance.

RESULTS: During placebo treatment, mean (± SD) epinephrine levels reached a peak of 431 ± 135 pmol/liter after 15 minutes of stress; the epinephrine response was blunted during estradiol treatment, with a peak of 357 ± 77 pmol/liter ($P < 0.05$). Estradiol also blunted the diastolic blood pressure response to stress (baseline levels of 78 ± 15 mm Hg vs peak of 90 ± 6 mm Hg during placebo; baseline of 80 ± 8 mm Hg vs peak of 84 ± 6 mm Hg during estradiol; $P < 0.05$). Estradiol treatment also blunted the decrease in the standard deviation of the mean of the electrocardiographic RR intervals and the increase in the ratio between the low-frequency and high-frequency bandwidths.

CONCLUSION: We observed a moderate, although significant, reduction in markers of the stress response to mental arithmetic in postmenopausal women treated with transdermal 17-beta-estradiol. **Am J Med. 2000;109:463–468.** ©2000 by Excerpta Medica, Inc.

Sex hormones affect the activity of the sympathoadrenal system. The catecholamine responses to various stimuli, such as physical exercise (1,2), hypoglycemia (3), and mental stress (1), are lower in women than in men. There is a reduction in the stress-induced catecholamine response in men after estrogen administration (4). Estrogens reduce catecholamine release from adrenomedullary cells (5) and affect the enzymatic pathways regulating catecholamine synthesis and degradation (6,7). They may also modulate the activity of both alpha- and beta-adrenoreceptors (8–10).

The menopause-related decline in circulating levels of estrogens is associated with an increased cardiovascular risk (11), and postmenopausal women have a greater cardiovascular response to certain stressful stimuli than do premenopausal women (12). However, although observational studies have reported a significant decrease in cardiovascular events in women who use postmenopausal estrogen therapy (13,14), a randomized trial found that estrogens were not effective for the secondary prevention of coronary heart disease in postmenopausal women (15). Moreover, interim results from the Women's Health Initiative, a primary prevention trial, have suggested a potential increase in the risk of heart disease during postmenopausal estrogen therapy (16). Therefore, although estrogens have beneficial effects on serum lipid levels and endothelial and vasomotor function (17,18), the overall effects of postmenopausal hormonal replacement therapy are uncertain.

One unsolved question is whether estrogens affect the cardiovascular system by decreasing the sympathoadrenal response to acute stress. Investigators have reported that estrogens reduce both basal and stress-induced sympathetic tone (19,20), but others have reported no effects on cardiovascular responses to stress (21,22). Different regimens, including dose, duration, and route of administration, of estrogen were used in these studies. Therefore, we studied the effects of 17-beta-estradiol, administered transdermally for 3 weeks, on plasma catecholamine levels and the cardiovascular and metabolic responses to a mental stress challenge in postmenopausal women.

MATERIAL AND METHODS

The study was performed in 10 postmenopausal women, who had a mean age of 53.9 ± 1.1 years, a mean body

From the Department of Internal Medicine and Biomedical Sciences (GC, MF, SM, IR, CM, GV), and the Institute of Gynecology (ABM, MR), University of Parma, Parma; and the Division of Cardiology, Regina Margherita Hospital (AA), Torino; and the Department of Internal Medicine, University of Modena (GDR), Modena, Italy.

Requests for reprints should be addressed to Graziano Ceresini, MD, Department of Internal Medicine and Biochemical Sciences, Section of Geriatrics, University of Parma, Via don Bosco 2, 43100 Parma, Italy.

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mass index of 25.2 ± 1.3 kg/m^2, and few or no vasomotor symptoms. All women had undergone natural (nonsurgical) menopause. Menopausal status was confirmed (serum levels of follicle-stimulating hormone greater than 40 mU/mL and estradiol less than 60 pmol/liter). All subjects were nonsmokers, had normal serum lipid levels, and had no history of cardiovascular disease. They had a low caffeine intake and took no medications. None had ever used estrogen replacement therapy. All of the subjects answered the Kellner Symptom Questionnaire (23), the Paykel Life Events Scale (24), and the Spielberg State-Trait Anxiety Inventory (25); those with identified psychological problems or who had experienced stressful life events in the previous 6 months were excluded. Subjects gave their informed consent to participate in the study. The protocol was approved by the Ethics Committee of the University of Parma.

Design

Subjects were treated with 17-beta-estradiol or placebo for 3 weeks, according to a randomized, double-blind, crossover design with a 3-week wash-out between the two treatments; 6 women were randomly assigned to receive estrogen as the first treatment. Both treatments were administered by skin patches (Rotta Research, Milan, Italy), which were renewed every 3 days. The release of 17-beta-estradiol was 50 μg per day. At the end of each treatment, the subjects underwent a mental stress test.

Mental Stress Test

In preliminary sessions, the subjects were familiarized with the serial subtraction test and the digit span test. Both sessions of mental stress were performed in the morning between 9 and 10 AM. The subjects ate a light breakfast at least 2 hours before the experiment, avoiding substances known to affect the sympathoadrenal system. They were seated in a comfortable chair in a quiet testing chamber. A 19-gauge intravenous catheter was placed in a superficial vein of the hand; the hand and the distal forearm were then placed in a box heated at a constant temperature of 55° C to obtain arterialized venous blood samples. The catheter was kept patent with a slow infusion of 0.9% saline. A cuff for automatic recording of blood pressure (A&D International, Ltd., Tokyo, Japan) was placed in the arm contralateral to the one used for blood sampling. In addition, continuous automatic electrocardiographic (ECG) monitoring was performed using a three-channel amplitude modulated tape recorder (Cardio Corder; Del Mar Avionics, Irvine, California). The stress test was divided into three consecutive parts: an initial 30-minute period of resting, a 15-minute mental stress, and a final 15-minute recovery period in resting conditions. During the last 15 minutes of the initial rest period, two (−15 and 0 minute) blood samples were collected, and systolic and diastolic blood pressures were recorded.

The 15-minute mental stress was divided into three 5-minute periods, each consisting of two different tasks: a 4-minute serial subtraction test and a 1-minute digit span test. The serial subtraction test required subjects to subtract 17 from a four-digit number. The digit span test required subjects to remember numbers in the correct sequence forward during the first and the third periods, and backward during the second period of the stress challenge. The length of the items was varied to make the difficulty of the task unpredictable. The numbers used were the same as those in the Digit Span Test of the Wechsler Adult Intelligent Scale (26). The subjects performed the tests as fast as possible and were accompanied by a metronome; they were not allowed to use paper or pencil during the procedure. If they made a mistake, they were asked to start again from the beginning. Blood samples were collected during the mental stress at 5, 10, and 15 minutes from the beginning of the challenge. At each sample time, systolic and diastolic blood pressures were recorded.

At the end of the stress challenge, subjects were left in resting conditions for 15 minutes, after which a blood sample was collected and blood pressure was recorded; soon after, the continuous automatic ECG monitoring was stopped. The subjects rated the overall difficulty of the tests using a five-point scale (with 1 indicating easy and 5 indicating very hard). To assess the level of anxiety caused by participation in the study, subjects completed the Spielberg State-Trait Anxiety Scale before and at the end of the stress challenge.

Measurements of Hormonal and Biochemical Variables in Blood Samples

Blood samples for measuring plasma epinephrine and norepinephrine levels were collected at each sample time in tubes containing glutathione and ethyleneglycol-bis-(beta-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) and immediately put on ice; after separation in a refrigerated centrifuge, plasma was stored at −80° C until assayed. Plasma catecholamine levels were evaluated using a reverse-phase high-performance liquid chromatography method with electrochemical detection (4). Blood glucose levels were determined with a glucose analyzer (Beckman, Palo Alto, California). Serum insulin levels were measured by radioimmunoassay (Sorin, Milan, Italy). Serum levels of free fatty acids were determined in basal conditions and at the end of both the 15-minute stress test and the recovery period by a spectrophotometer (Beckman, Palo Alto, California). Serum levels of 17-beta-estradiol were evaluated on basal samples that were collected in both sessions before the stress test by radioimmunoassay using a commercially available kit (Sorin, Milan, Italy). All samples were run in duplicate. The intra- and interassay coefficients of variation were less than 10% for all of the measurements.

Analysis of Continuous ECG Recording

Three leads were used for monitoring. The Strata Scan Holter Analysis System program (Del Mar Avionics, Irvine, California) was used for the tape analysis. Heart rate was expressed as the mean of the RR intervals. A histogram of the consecutive RR ratio was examined, and cycles outside 80% to 120% of preceding RR intervals were excluded to avoid the interference of artifacts, premature beats, or postextrasystolic pauses (27,28). All tapes were then analyzed to measure heart rate variability in both the time and frequency domains. Recordings were analyzed by one investigator who was unaware of treatment. In the time domain of heart rate variability, we calculated the standard deviation of the mean of all normal RR intervals in the following three periods: the final 15 minutes of the initial resting conditions, the 15-minute stress challenge, and the 15-minute recovery period. In the same intervals, we also evaluated the frequency domain of heart rate variability; spectral measurements were computed by fast-Fourier transform analysis. Spectral plots were used to identify two subsets of the frequency domain: low frequency (0.05 to 0.15 Hz) and high frequency (0.15 to 1.35 Hz). Spectral power was quantified in these two frequency bandwidths. Spectral plots were squared to quantify power in the two frequency bands (in milliseconds squared). The ratio of low-frequency to high-frequency power was calculated.

Data Analysis

A preliminary analysis with the Shapiro-Wilk W statistic test was performed to determine whether the data conformed to a normal distribution, and the homogeneity of variance was computed by Bartlett's test. Data were analyzed using repeated measures analysis of variance in which the effects of both time and treatment were evaluated. There were no statistically significant interactions between order of treatment and effects on stress response (all $P < 0.19$); thus results were combined. If an F value was significant ($P < 0.05$), Student's t test was used to compare means between the groups. When data were not normally distributed, analysis was performed using the Friedman rank test followed by the Wilcoxon signed rank test to identify differences between distributions; these tests were used to evaluate the effects of treatment on free fatty acid levels. All statistical calculations were made using SPSS software (SPSS, Chicago, Illinois) (29). Continuous values are expressed as mean ± SD.

RESULTS

Serum estradiol levels were increased at the end of the treatment with the 17-beta-estradiol patch (placebo 30 ± 8 pmol/liter vs estradiol 171 ± 25 pmol/liter, $P < 0.003$). Baseline Spielberg State Anxiety scores were similar during the two treatments (placebo 40 ± 3 vs estradiol 43 ± 5, $P = 0.72$) and were not affected by the mental stress challenge (placebo, 41 ± 4; estradiol 41 ± 5, $P = 0.61$). The subjects rated the test as being equally difficult (on a 1-to-5 scale) during both the placebo and the estradiol sessions (placebo 3 ± 0.6 vs estradiol 3 ± 1, $P = 0.21$).

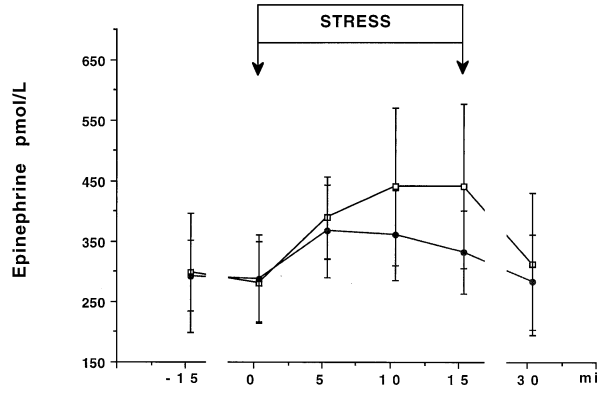

Figure 1. Mean (± SD) plasma epinephrine levels before, during, and after mental stress after 3-week treatment with placebo (open square) or estradiol (filled circle) in 10 postmenopausal women. Repeated measures analysis of variance revealed a significant ($P < 0.05$) difference between treatments.

Evaluations of Circulating Levels of Catecholamines and Other Hormonal and Biochemical Variables

Basal plasma epinephrine levels were similar during the estradiol and placebo treatments. In response to the mental stress (Figure 1), epinephrine levels increased significantly during placebo treatment, from basal values of 273 ± 67 pmol/liter to a peak of 431 ± 135 pmol/liter measured at the end of the stress challenge. The epinephrine response was less marked during estradiol treatment. From a baseline of 279 ± 72 pmol/liter, the values reached a peak of 357 ± 77 pmol/liter ($P < 0.05$ compared with baseline) 5 minutes after the beginning of stress, with a subsequent continuous decrease. There was a significant ($P < 0.05$) difference in the effects of placebo and estradiol on the stress-induced epinephrine responses using repeated measures analysis of variance.

There were no significant differences in basal levels of plasma norepinephrine during the two treatments. In response to the stress stimulus, norepinephrine levels increased during both treatments; however, these changes were not statistically significant (Figure 2).

Basal serum glucose and insulin levels were similar during the two treatments and were not significantly affected by the mental stress challenge during either placebo or 17-beta-estradiol treatment. Serum free fatty acid levels increased significantly in response to the stress challenge during the administration of both placebo [peak values of 667 ± 163 μM vs basal values of 520 ±

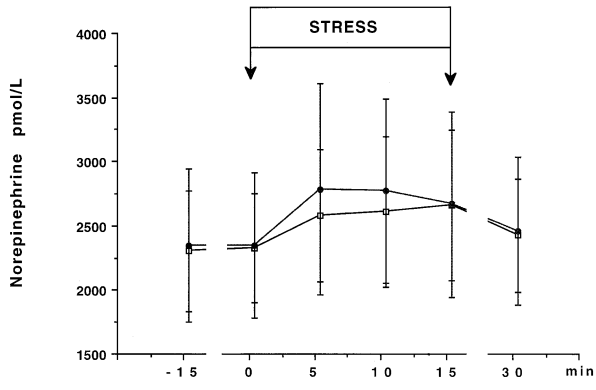

Figure 2. Mean (± SD) plasma norepinephrine levels before, during, and after mental stress after 3-week treatment with placebo (open square) or estradiol (filled circle).

136 μM ($P < 0.05$)] and 17-beta-estradiol (peak values of 617 ± 123 μM vs basal values of 489 ± 108 μM ($P < 0.05$); Figure 3]. There were no differences between treatments in either the absolute increments ($P = 0.67$) or the area under the curve responses for free fatty acids levels ($P = 0.70$).

Evaluations of Blood Pressure Levels and Continuous ECG Monitoring

Basal systolic and diastolic blood pressures were similar during the two treatments (Figure 4). In response to mental stress, systolic blood pressure increased significantly during placebo treatment, from 120 ± 9 mm Hg to a peak of 136 ± 15 mm Hg ($P < 0.001$). During estradiol treatment, systolic blood pressure also increased significantly in response to the stress challenge, from 119 ± 9 mm Hg to a peak of 133 ± 15 mm Hg ($P < 0.01$). There were no effects of treatment on the stress-induced systolic blood pressure changes. However, estradiol treatment

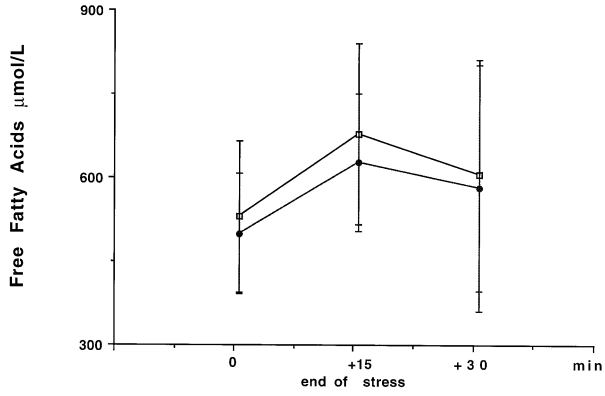

Figure 3. Mean (± SD) serum free fatty acid levels evaluated at baseline, at the end of mental stress, and at the end of the recovery period after 3-week treatment with placebo (open square) or estradiol (filled circle).

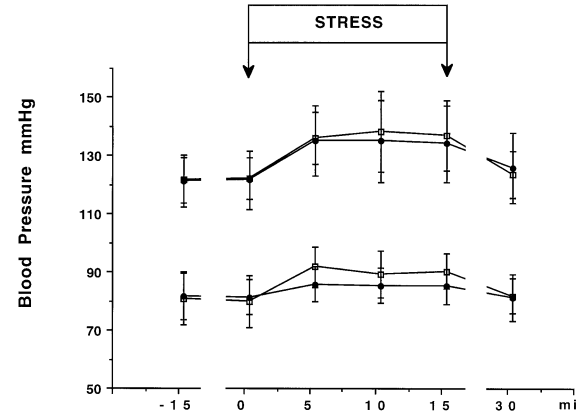

Figure 4. Mean (± SD) systolic blood pressure (above) and diastolic blood pressure (below) before, during, and after mental stress after 3-week treatment with placebo (open square) or estradiol (filled circle). Repeated measure analysis of variance revealed a significant ($P < 0.05$) difference between treatments in the diastolic blood pressure response.

blunted the effects of stress on diastolic blood pressure ($P < 0.05$). Mean diastolic blood pressure was significantly increased from baseline during placebo treatment (peak levels of 90 ± 6 mm Hg vs basal levels of 78 ± 15 mm Hg, $P < 0.002$), but not during estrogen treatment (peak levels of 84 ± 6 mm Hg vs basal levels of 80 ± 8 mm Hg, $P = 0.64$).

Subjects had similar mean RR intervals, standard deviation of all mean RR intervals, and low-frequency and high-frequency bandwidths during the two treatments (Table). Mean RR intervals decreased significantly during the stress challenge during both treatments; the decrement was somewhat less pronounced during estradiol treatment, but the difference between treatments was not statistically significant ($P = 0.51$). In response to stress, there was a significant decrease in the standard deviation of all mean RR intervals values ($P < 0.01$) during placebo treatment, but not during estrogen treatment ($P = 0.23$, $P < 0.05$ for the comparison of placebo and estradiol treatments). We observed a stress-related increase in low-frequency bandwidths during both treatments; there were no effects of treatment on stress-induced high-frequency bandwidths. The ratio of low-frequency to high-frequency bandwidths increased after the stress challenge during both treatments but were significantly lower during estradiol treatment ($P < 0.05$).

CONCLUSION

We found that transdermal administration of 17-beta-estradiol reduced the effects of mental arithmetic stress on plasma epinephrine levels and diastolic, but not systolic, blood pressure in postmenopausal women. These

Table. Indexes of Heart Rate and Heart Rate Variability before, during, and after Mental Stress after 3-Week Treatment with Either Placebo or Estradiol in 10 Postmenopausal Women

Measurement	Placebo			17-beta-Estradiol		
	Baseline	Stress	Recovery	Baseline	Stress	Recovery
	Mean ± SD					
Mean of all RR intervals (msec)	828 ± 39	776 ± 42	816 ± 56	824 ± 35	777 ± 37	831 ± 51
Standard deviation of all mean RR intervals (msec)	59 ± 11	47 ± 7	50 ± 9	54 ± 15	47 ± 12	52 ± 23
Low-frequency bandwidth ($msec^2$)	494 ± 172	1,562 ± 1,046	699 ± 269	543 ± 154	756 ± 152*	551 ± 250
High-frequency bandwidth ($msec^2$)	327 ± 133	309 ± 200	310 ± 120	320 ± 157	238 ± 121	276 ± 188
Low-frequency/high-frequency ratio	1.9 ± 0.6	5.9 ± 2.3	2.7 ± 1.4	2.1 ± 1	3.7 ± 1.4*	2.4 ± 0.9

*$P < 0.05$ versus placebo, by analysis of variance.

effects were accompanied by a decrease in the responses of some measures of stress-induced cardiac sympathetic tone. The blunting effects of estrogen administration are unlikely to be the result of a learning effect on mental stress, given that we used a double-blind, crossover design. Moreover, the Spielberg State Anxiety scores and the degree of difficulty of the mental stress were similar during the two testing sessions. Thus, it seems unlikely that the reduced sympathoadrenal response to stress that we observed during the estradiol treatment was primarily the result of the stress being perceived as less severe.

Although estradiol had a moderate effect in reducing stress-induced plasma epinephrine levels, it did not have a significant effect on norepinephrine levels. However, norepinephrine levels were not significantly increased during mental stress with either treatment. These observations agree with previous findings that there is less activation of the sympathetic nervous system (ie, norepinephrine levels) than of the adrenal medulla (ie, epinephrine levels) during mental stress (30,31). In our study, subjects were tested at the end of 3 weeks of 17-beta-estradiol administration. Estrogens rapidly and directly inhibit catecholamine secretion from the adrenal medulla, likely through a nongenomic mechanism (32). Thus, we believe that 3 weeks of estradiol administration was sufficient to detect any effects of treatment on stress-induced adrenomedullary activity.

We did not find any significant effects of estrogen administration on stress-induced heart rate, as measured by mean RR intervals. However, when the heart rate variability was evaluated in the time domain, estradiol blunted the stress-related decrease in the standard deviation of all mean RR intervals. Similarly, estradiol reduced the response of the low-frequency/high-frequency ratio to stress, primarily because of a decrease in the values of the low-frequency bandwidth. The changes are directly related to the cardiac sympathetic tone, both in basal conditions and during psychologic stress (33,34). Our results, therefore, suggest that a decrease in the stress-induced cardiac sympathetic activity occurs during 17-beta-estradiol supplementation in postmenopausal women.

Recently, Komesaroff et al (35) reported a reduction in the blood pressure and catecholamine responses to mental stress during oral estrogen administration in perimenopausal women. We could only partially confirm those results, as we found that estradiol had a less potent blunting effect on the overall sympathoadrenal response to stress. Therefore, we hypothesize that a reduction in the sensitivity of the sympathoadrenal system to estrogen occurs during the transition from perimenopause to overt postmenopausal status. We studied postmenopausal women who had no or minor vasomotor symptoms, to avoid the potential bias of an estrogen-related relief in those symptoms. This may have led to a less severe perception of the stress stimulus during estrogen treatment. However, in the study by Komesaroff et al, estrogen administration resulted in circulating estradiol levels greater than those that occur during the standard transdermal estrogen administration, as in our study. Therefore, the more complete blunting effect of estrogen on mental stress–induced sympathoadrenal activity in the previous study could have been the result of greater circulating estrogen levels.

In agreement with a previous report (36), we did not find any stress-related changes in either blood glucose or insulin levels, but we did observe similar, statistically significant mental stress–induced increases in serum levels of free fatty acids during both placebo and estradiol treatment. Mental stress causes an increase in catecholamine-mediated lipolytic activity through an activation of beta-adrenoreceptors (37), and estradiol decreases the stress-induced activation of lipolysis in men (4). Data from experimental animals show that estrogens blunt the beta-

adrenoreceptor response to catecholamine stimulation (10). Thus, postmenopausal estrogen supplementation might be expected to reduce the effects of mental stress–induced sympathoadrenal activation on lipolysis; however, our results did not confirm this hypothesis.

In conclusion, we found that transdermal 17-beta-estradiol administration, at a dose of 50 μg per day, moderately, but significantly, reduces the response to mental stress, as measured by plasma epinephrine levels, diastolic blood pressure, and the overall cardiac sympathetic tone, in postmenopausal women. Studies are needed to determine the influence of estrogens, either alone or in combination with progestins, on sympathoadrenal function in basal conditions, during daily activities, and after challenges with several types of psychologic tests.

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