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Estrogen Supplementation Attenuates Glucocorticoid and Catecholamine Responses to Mental Stress in Perimenopausal Women*

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ABSTRACT

Estrogens are reported to provide protection against the development of cardiovascular disease in women, but the mechanisms underlying these effects are not well defined. We hypothesized that estrogen might affect the hormonal responses to stress. We therefore studied cortisol, ACTH, epinephrine, norepinephrine, and norepinephrine spillover and hemodynamic responses to a 10-min mental arithmetic test in 12 perimenopausal women randomized to 8 weeks of estrogen supplementation (estradiol valerate, 2 mg daily; n = 7) or placebo (n = 5). Total body and forearm norepinephrine spillover were measured by radiotracer methodology. After supplementation with estradiol, the increases in both systolic and diastolic blood pressure in response to mental stress were reduced, and cortisol, ACTH, plasma epinephrine and norepinephrine, and total body norepinephrine spillover responses to stress were significantly attenuated ($P < 0.05$ in each case). Forearm norepinephrine spillover was unchanged by estrogen, and there was no change in any of the responses after placebo. We conclude that estrogen supplementation in perimenopausal women attenuates blood pressure, glucocorticoid, and catecholamine responses to psychological stress. (*J Clin Endocrinol Metab* **84:** 606–610, 1999)

OBSERVATIONAL studies of chronic estrogen therapy in postmenopausal women show a significant reduction in the risk of coronary events, strongly suggesting that estrogens exert cardioprotective effects (1). The precise mechanisms underlying such effects, however, remain unclear.

In recent studies in perimenopausal women we have shown that estrogen administration attenuates total body norepinephrine (NE) spillover and vascular responses to intraarterial NE and increases basal endothelial nitric oxide release (2, 3). Young women have been shown to have reduced hormonal responses to psychological stress compared with young men (4–6), although their cardiovascular responses to stress have been reported to be greater (7, 8). Some evidence exists from previous studies that estrogen blunts stress responses in postmenopausal women (9).

Psychological stress results in increases in adrenocortical hormones and catecholamines, both of which potentially influence cardiovascular reactivity. Further, mental stress may be related to risk factors for coronary artery disease (10, 11) or directly to cardiovascular events (12). Accordingly, one of the factors contributing to the apparent beneficial actions of estrogens on the cardiovascular system may include modulation of the stress response.

In this placebo-controlled study, we examined the effects of 8 weeks of estrogen supplementation on the hemodynamic and hormonal responses to 10 min of psychological stress in healthy perimenopausal women, chosen because of the clinical impression that stress responses are increased in this group. Plasma levels of cortisol (F), ACTH, epinephrine (EPI), and NE and total body and forearm NE spillover were measured at baseline, during stress, and at the end of stress.

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Subjects and Methods

Twelve perimenopausal women were recruited through advertisements in a local newspaper. All were within 2 yr of their last period and were actively experiencing vasomotor symptoms of menopause. Women with one or more cardiovascular risk factors, with clinical evidence of vascular disease, or who were taking vasoactive medications were excluded. The study was approved by the Alfred Hospital ethics committee, and all subjects gave written fully informed consent.

Subjects were randomized to receive 8 weeks of either estrogen supplementation as estradiol valerate (Progynova, Schering, Alexandria, Australia; 2 mg daily; n = 7) or placebo (n = 5); sample sizes were chosen on the basis of previous studies (2, 3, 13) to achieve a power of 80% to detect a difference of 20% in the response variables with a type 1 error of 0.05. Hemodynamic studies and assessment of forearm vascular reactivity were performed on two separate occasions, 8 weeks apart. Subjects were unaware of the treatment they were receiving, and all measurements of hormone levels and NE kinetics were made by investigators who were blinded to the treatment regimen. On each study day, subjects underwent the following procedures.

Arterial cannulation

Subjects rested in the supine position throughout each study in a quiet temperature-controlled room maintained at 22 C. The brachial artery of the left arm was then cannulated with a 21-gauge, 5-cm catheter (Cook, Brisbane, Australia) under strict aseptic conditions after local anesthesia (1% lignocaine, Astra, Sydney, Australia) for intraarterial measurement of blood pressure (Spacelabs, Inc., Washington DC), and arterial blood sampling. Heart rate was continuously monitored by electrocardiography. After brachial cannulation, subjects rested for 30 min before commencement of the study.

Psychological stress test

The stress was administered as a standard mental arithmetic test as previously described (13). In brief, subjects were asked to perform a series of subtractions and additions, accompanied by a repetitive annoying background noise to induce difficulty in concentration.

Blood sampling

Simultaneous venous and arterial blood samples were taken at baseline and at 5 and 10 min after commencement of stress. An additional blood sample was taken at 25 min.

Measurements of F, ACTH, and estrogen levels

Assays for F and ACTH were carried out on the baseline, 5 min, and 10 min samples and for ACTH on the 25 min sample on both days. Baseline samples were also assayed for estradiol. ACTH was adsorbed from plasma onto porous Vycor glass and measured by specific RIA as previously described (14). The material was a gift of Dr. Rolf Gaillard, Association of Trade with America, Geneva, Switzerland. The interassay coefficient of variation was 7% (n = 4), and the sensitivity was 15 pg/mL. F was measured in unextracted plasma by specific RIA. The intraassay coefficient of variation was 8% (n = 32), and sensitivity was 10 nmol/L. Estradiol was also measured by RIA. The intraassay coefficient of variation was 9% (n = 32), and the sensitivity was 30 pmol/L.

Assay of endogenous and radiolabeled catecholamines

Blood samples were transferred immediately to ice-chilled tubes containing ethyleneglycol-bis-(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid and reduced glutathione and were centrifuged at 4 C. The plasma was stored at −70 C before assay (always within 2 months). Plasma concentrations of endogenous NE were determined by high performance liquid chromatography with electrochemical detection, as previously described (2). The intraassay coefficient of variation was 8%, the interassay coefficient of variation was 9% (n = 10), and the sensitivity of the assay was 12 pg/mL. Plasma [^{3}H]NE was assayed by liquid scintillation counting.

Assessment of total body NE clearance and spillover

Total body NE clearance and spillover to plasma were measured by a radiotracer method, as previously described (2). This method involves the continuous iv infusion of a tracer dose of tritiated NE (Levo-7 [^{3}H]NE, New England Nuclear, Boston, MA; 0.7 μCi/min; SA, 12–20 Ci/mmol) to a steady state concentration in plasma. The total NE spillover to plasma, and the total plasma NE clearance rate can then be calculated as follows: total body NE clearance = [^{3}H]NE infusion rate (dpm/min)/plasma [^{3}H]NE concentration, and total body NE spillover = [^{3}H]NE infusion rate/plasma NE specific activity (dpm/pg), where dpm is the disintegrations per min of [^{3}H]NE.

Assessment of forearm NE spillover

Forearm spillover rates were calculated according to the Fick principle, with adjustment for NE uptake across the forearm, using the fractional extraction of [^{3}H]NE, as previously described (13): fractional extraction of [^{3}H]NE across the forearm = [(arterial [^{3}H]NE) − (venous [^{3}H]NE)]/(arterial [^{3}H]NE), and forearm NE spillover = [(NEv − NEa) + (Nea × NEex)] × FPF, where NEa and NEv are the NE concentrations in the arterial and venous effluent plasma, NEex is the fractional extraction of [^{3}H]NE in a single passage through the forearm, and FPF is the forearm plasma flow (milliliters per min).

Calculations and statistical analysis

Results are expressed as the mean ± SEM. Stress response curves were compared by two-way repeated measures ANOVA; where data were not normally distributed, analysis was undertaken using Friedman's repeat measures ANOVA on ranks with *post-hoc* analysis conducted using Dunnett's method. Other data were compared by Student's *t* test after application of a modified Levene procedure to assess homogeneity of variance. Where the sample variance ratio was greater than 4, a log transformation was performed before the application of this test. Where multiple comparisons were made, an appropriate Bonferroni correction factor was applied. Statistical testing was carried out using the SigmaStat software program. The null hypothesis was rejected at $P < 0.05$.

Results

Subject characteristics

The mean age of the subjects receiving estrogen was 48 ± 2 yr, and that of those receiving placebo was 50 ± 2 yr. Mean weights were 49.8 ± 2.2 and 63.6 ± 2.2 kg, respectively. Although all women were actively experiencing menopausal symptoms and all showed FSH levels clearly in the menopausal range, estradiol levels varied between 25–140 pmol/L, indicating some variability in hormonal status at baseline.

Effect of estrogen supplementation on basal estradiol, FSH, F, ACTH, EPI, and NE levels and blood pressure (Table 1)

No significant change was detected in basal ACTH, F, EPI, or NE levels with either E or placebo. Estradiol levels increased in subjects receiving estrogen ($P < 0.008$), but there was no change in subjects receiving placebo.

Effects of estrogen on blood pressure, heart rate, and forearm blood flow responses to stress

Estrogen significantly attenuated the increases in both systolic and diastolic pressures induced by mental stress ($P <$

TABLE 1. Hemodynamic and biochemical variables at baseline (Pre) and after treatment (Post) with either estrogen (n = 7) or placebo (n = 5)

	Estrogen group		Placebo group	
	Pre	Post	Pre	Post
Estradiol (pmol/L)	121 ± 41	1118 ± 111[a]	107 ± 22	115 ± 19[b]
FSH (U/L)	75 ± 22	52 ± 14	81 ± 15	80 ± 13
Cortisol (nmol/L)	300 ± 55	271 ± 41	207 ± 43	210 ± 55
ACTH (pg/mL)	17 ± 3	17 ± 4	16 ± 4	20 ± 4
EPI (pg/mL)	109 ± 30	80 ± 28	49 ± 10	61 ± 18
NE (pg/mL)	341 ± 62	222 ± 17	169 ± 37	178 ± 42
SBP (mm Hg)	133 ± 3	125 ± 2	130 ± 2	129 ± 1
DBP (mm Hg)	83 ± 2	76 ± 1	79 ± 2	78 ± 1

Values are the mean ± SEM. SBP and DBP, Systolic and diastolic blood pressures, respectively.
[a] Significant difference from pretreatment variable ($P < 0.008$).
[b] Significant difference from corresponding value in estrogen-treated group ($P < 0.03$).

0.001; see Fig. 1). However, there was no effect on the increases in heart rate or forearm blood flow (data not shown).

Effect of estrogen on ACTH and F responses to stress

Mean responses to mental stress before and after E and before and after placebo are shown for ACTH and F in Fig. 2. After estrogen supplementation, the F responses were markedly attenuated, as measured by two-way ANOVA with repeated measures ($P = 0.02$); areas under the curves and changes from baseline were also reduced according to *t* test ($P < 0.05$). ACTH responses were reduced according to Friedman repeated measures ANOVA on ranks ($P < 0.05$) and as measured by areas under the curves ($P = 0.04$). There was no change in either the ACTH or F response after placebo.

Effect of estrogen on EPI and NE responses to stress

Mean arterial EPI and NE responses to mental stress before and after E and before and after placebo are shown in Fig. 3. NE responses were attenuated according to two-way repeated measures ANOVA ($P = 0.03$), and EPI responses were reduced according to repeated measures ANOVA on ranks ($P < 0.05$). No significant differences were detected after treatment with placebo.

Effect of estrogen on total body and forearm NE spillover responses to stress

Mean responses of total body and forearm NE spillover to mental stress before and after E and before and after placebo are shown in Fig. 4. As previously reported (2), basal total body NE spillover was reduced after E treatment, and the levels remained reduced throughout the stress. Total NE spillover was reduced after E treatment according to two-way repeated measures ANOVA ($P = 0.04$); however, there was no effect on forearm NE spillover, and no significant differences were detected after placebo.

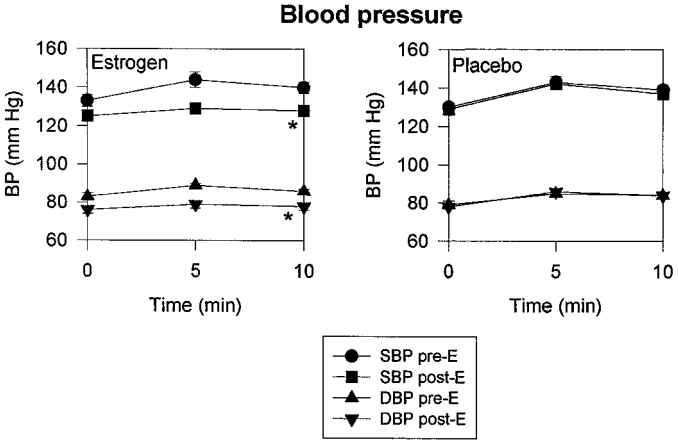

FIG. 1. Blood pressure responses to 10 min of psychological stress before and after 8 weeks of treatment with either estrogen or placebo. *, Both systolic and diastolic blood pressure responses were significantly attenuated ($P < 0.001$) after estrogen treatment. Responses were unchanged after placebo treatment.

Discussion

This study demonstrates that 8 weeks of estrogen supplementation attenuates the responses to 10 min of psychological stress of systolic and diastolic blood pressure, F, ACTH,

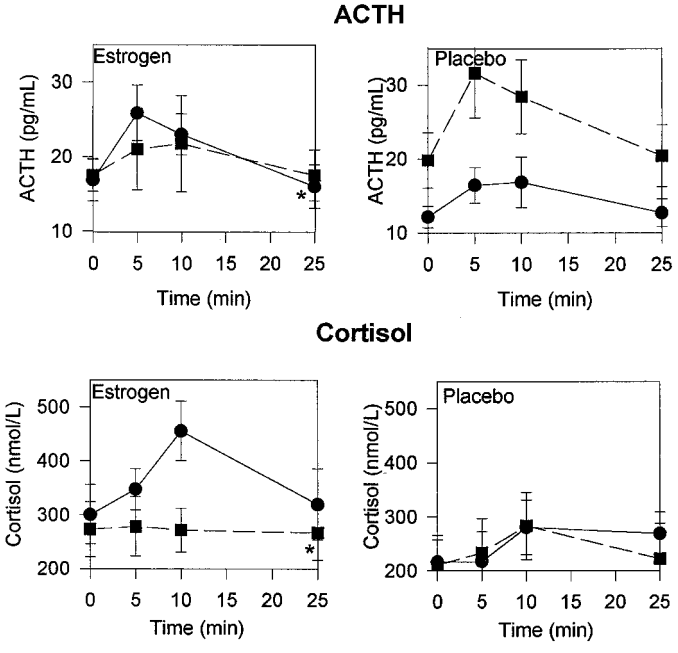

FIG. 2. ACTH and F responses to 10 min of psychological stress before (*circles*) and after (*squares*) treatment with estrogen or placebo. *, Both sets of responses were reduced after E treatment, as measured by repeated measures ANOVA as described in *Materials and Methods* ($P < 0.05$ and $P = 0.02$, respectively). No significant differences were detected after treatment with placebo.

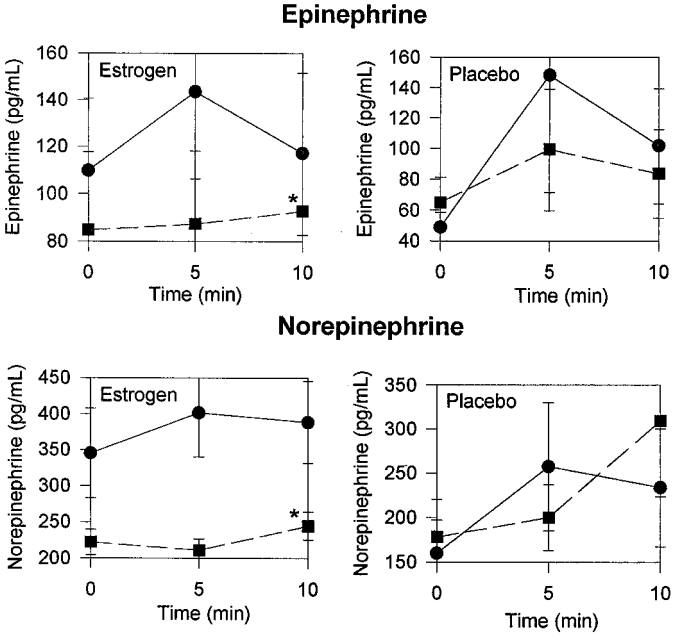

FIG. 3. Mean arterial EPI and NE responses to 10 min of psychological stress before (*circles*) and after (*squares*) treatment with estrogen or placebo. *, Both sets of responses were reduced after E treatment, as measured by repeated measures ANOVA as described in *Materials and Methods* ($P < 0.05$ and $P = 0.03$, respectively). No significant differences were detected after treatment with placebo.

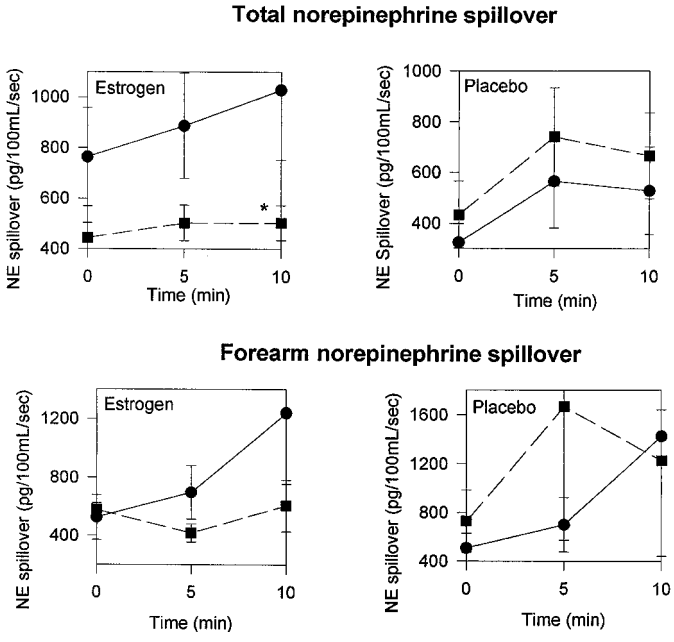

FIG. 4. Total body and forearm NE spillover responses to 10 min of psychological stress before (*circles*) and after (*squares*) treatment with estrogen or placebo. *, Total body NE spillover response was reduced after E treatment ($P = 0.04$). However, there was no change in forearm NE spillover, and no significant differences were detected after placebo.

EPI, NE, and total body NE spillover. However, estrogen appears to have no effect on heart rate, forearm blood flow, or forearm NE spillover responses. No significant change occurred in basal levels of ACTH, EPI, NE, or total F with either E or placebo (free F was not assessed).

We have previously shown that in perimenopausal women, estrogen supplementation enhances basal nitric oxide release (3) and attenuates both total body NE spillover and NE-induced vasoconstriction (2). As in the present study, these changes were associated with a reduction in resting blood pressure. The attenuation of stress-induced increases in both systolic and diastolic blood pressure in this study further highlights the hemodynamic effects of estrogen supplementation.

Our results are consistent with those of previous studies, which have suggested that cardiovascular, glucocorticoid, and catecholamine responses to stress vary between males and females. The cardiovascular responses of young women to psychological stress appear to be greater than those of young men (5) and to be enhanced after E administration (15). Women have been shown to have a reduced EPI response to mental stress compared with men (7), and female college students have smaller F, EPI, and NE responses to examination stress and other behavioral stressors than do male students (4–6), although in these studies urinary catecholamine excretion, but not plasma levels or spillover, were measured, making the results difficult to interpret. Attention has been drawn to the possible role of sex steroids in modulating social, psychological, and metabolic variables (4, 5, 7, 9). Cardiovascular responses to stress are increased after menopause (16), and studies conducted on women during the menstrual cycle have shown variations in the stress response (17). For example, in one study in normal women the luteal phase was associated with greater stroke volume responses and lower vascular tone than those during the follicular phase (8); in another, women in the luteal phase reacted significantly more to a cold pressor test, but not to mental arithmetic (18); and in another, the responses of systolic blood pressure and pulse rate to normal environmental stressors in cycling young women were greater in the luteal phase (19). Further, in an experimental study in intact ewes we have shown that both glucocorticoid and catecholamine responses to stress vary during the estrous cycle (20).

In studies in young women it is difficult to distinguish between the effects of estrogens and those of progesterone. One previous study (9) addressed the question of the effect of estrogen administration on hormonal responses to stress in menopausal women. After 6 weeks of transdermal estrogen treatment, the maximum percent changes in ACTH, F, NE, and androstenedione failed to reach significance, suggesting a blunting of these responses. In this study EPI responses were not reported, and catecholamine spillover studies to assess the relative balance between NE release and clearance were not conducted. Another study, in young men, showed that E administration blunts the EPI and NE responses to mental stress (21). In a further experimental study in sheep we have shown that estrogen administration after ovariectomy attenuates the F and ACTH responses to audiovisual and hypoglycemic stress (20). These results support those of the present study, which suggest that estrogens may play a role in limiting the responses to psychological stress.

Both adrenocortical hormones and catecholamines can influence vascular reactivity, which is of importance in determining cardiovascular risk. Further, mental stress during daily life has been shown to more than double the acute risk of myocardial ischemia (10); adolescents who have a greater cardiovascular response to mental stress compared to a control population have a greater risk of subsequently developing essential hypertension (11); and in patients with coronary artery disease, mental stress-induced myocardial ischemia is associated with significant increases in risks of fatal and nonfatal cardiac events (12). Accordingly, the effects we have demonstrated may contribute to the apparent protective effects of E with respect to cardiovascular risk. As the acute symptoms of menopause commonly include increased anxiety and irritability, it is also possible that a reduction in the stress response accounts for part of the beneficial effect of E on menopausal symptoms. It should be noted, however, that in the current study E was given for an 8-week period only. It is uncertain whether the effects we have observed would persist over a longer period of estrogen use and whether they would be modified by other clinical approaches to hormonal therapy, such as the concomitant use of a progestin.

Although the mechanisms of the effects of E on glucocorticoid levels are not yet fully defined, it appears probable that it acts via ACTH, and thus the pituitary or hypothalamus, rather than directly on the adrenal gland. This is consistent with evidence obtained from women with hypothalamic amenorrhea, in whom a blunted response to CRH administration and increased F levels were observed (22). These effects

could in part be explained by a direct action of estrogen on CRH gene expression; it is also likely that they are modulated at least in part by changes in glucocorticoid receptor (GR) number and/or function. In rats, it has been shown that estradiol abolishes the autologous down-regulation of GR seen in the hippocampus and hypothalamus (23). Further, there is evidence of gender-specific differences in the gene expression of hippocampal and hypothalamic GR and of an effect of exogenous E on GR messenger ribonucleic acid levels (24).

The mechanisms by which estrogens affect catecholamine levels are also uncertain. The bulk of circulating EPI appears to originate from the adrenal medulla, and that of NE from sympathetic neurons, although under conditions of stress, adrenal secretion of the latter increases, predominantly under neuronal control (25). Accordingly, both direct effects of E on the adrenal gland and effects on the central or peripheral nervous system are possible. Neurological pathways seem more likely (26, 27), although several different actions may be involved; it is established that estrogens increase the affinity of α_1-adrenergic receptors (28), and they may also affect β-receptor number and affinity (29); there is evidence that E modulates catecholamine synthesis in neural tissue (30), and increased urinary catecholamine secretion after E administration suggests that an effect on clearance is also possible (31). Whatever the mechanisms of action of estrogens on both glucocorticoid and catecholamine pathways, a complex relationship among hormonal effects, amelioration of symptoms, and stress responses is likely.

In conclusion, this study has shown that E supplementation in perimenopausal women in standard doses attenuates the pressor, glucocorticoid, and catecholamine responses to stress. These effects could explain part of the apparent beneficial actions of E both on acute symptoms of menopause and on long term cardiovascular risk.

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