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Short-Term Estradiol Treatment Enhances Pituitary-Adrenal Axis and Sympathetic Responses to Psychosocial Stress in Healthy Young Men*

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ABSTRACT

Evidence from animal studies and clinical observations suggest that the activity of the pituitary-adrenal axis is under significant influence of sex steroids. The present study investigated how a short term elevation of estradiol levels affects ACTH, cortisol, norepinephrine, and heart rate responses to mental stress in healthy men. In a double blind study, 16 men received a patch delivering 0.1 mg estradiol/day transdermally, and age- and body mass index-matched control subjects received a placebo patch. Twenty-four to 48 h later, they were exposed to a brief psychosocial stressor (free speech and mental arithmetic in front of an audience).

In response to the psychosocial stressor, ACTH, cortisol, norepinephrine, and heart rate were increased in both experimental groups (all $P < 0.0001$). However, the estradiol-treated subjects showed exaggerated peak ACTH ($P < 0.001$) and cortisol ($P < 0.002$) responses compared to the placebo group. Also, the norepinephrine area under the response curve was greater in the estradiol group ($P < 0.05$). Although heart rate response differences failed to reach statistical significance, they, too, tended to be larger in the estradiol group. Neither mood ratings before or after the stressor, nor ratings of the perception of the stressor could explain the observed endocrine response differences. In conclusion, short term estradiol administration resulted in hyperresponses of the pituitary-adrenal axis and norepinephrine to psychosocial stress in healthy young men independent of psychological effects, as assessed in this study. (*J Clin Endocrinol Metab* **81:** 3639–3643, 1996)

SEX DIFFERENCES in the adrenocortical response to moderate psychosocial stress have been consistently observed in our laboratory. Healthy men showed a 1.5- to 2-fold higher free cortisol response to a free speech and mental arithmetic task of 10-min duration than age-matched women (1, 2). In these studies, neither mood nor subjective stress ratings could explain the lower cortisol levels in women. Evidence accumulated that female sex steroids could play a crucial role in mediating the adrenocortical response to external stimulation, as a significant response difference was also observed in women using estrogen-containing oral contraceptives (OC). In several independent studies, OC users showed a further reduction in free cortisol levels after psychosocial stress (3) or physical stress (4, 5) compared to women who had never used OC.

The idea that estrogens are potent regulators of the pituitary-adrenal axis is not new. In fact, an extensive literature exists on the various effects of estrogens on these systems in animals. However, the results are heterogeneous and contradictory. Early reports by Kitay (6, 7) suggested that estradiol elevates basal corticosterone levels and enhances ACTH and corticosterone responses to various stimuli in rats. More recent studies appear to support these findings of enhanced pituitary-adrenal activity due to estradiol in different animal species (8–14).

The present experiment intended to investigate pituitary-adrenal axis and sympathetic responses to a potent psychosocial stressor in healthy men treated with estradiol. A cross-sectional study design and a control group matched for age and body mass index were chosen to avoid confoundation of steroid treatment and stress experience. Assessments of mood and subjective stress responses were included to possibly explain endocrine response differences.

Subjects and Methods

Subjects

Thirty-two men were recruited for participation in this study among students at the University of Trier. Before entering the study they were medically examined for current or past health problems to exclude subjects with psychiatric, endocrine, cardiovascular, or other chronic diseases. They reported to be nonsmokers and not currently taking medications. No biochemical analyses were performed to validate the individual's drug status. The participants were then randomly assigned to one of two treatment groups, *i.e.* estradiol (n = 16) and placebo (n = 16), respectively. Written informed consent was obtained from each subject. Participants received a compensation of 75 Deutsch marks upon completion of the experiment. The study protocol was approved by the ethics committee of the University of Trier.

Hormonal treatment

After the medical exam, subjects either received an estradiol (Estraderm TTS 100, Geigy, Wehr, Germany) or placebo patch, which was attached to the back in a double blind procedure. The estradiol patch delivers a mean of 100 μg estradiol/day percutaneously over 3–4 days.

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Experimental protocol

Twenty-four to 48 h after they received the patch, subjects returned to the laboratory to participate in the stress session. A catheter was inserted in an anticubital vein at −45 min followed by a 35-min rest period. At −10 min, the first blood sample and a saliva sample were obtained. A brief mood-rating scale (see below) was completed at −3 min, followed by second blood and saliva samples at −1 min. Then, the subjects were exposed to the Trier Social Stress Test (15), which mainly consists of a free speech and mental arithmetics performed in front of an audience. In the past, this stress protocol was found to reliably induce endocrine, cardiovascular, and subjective responses indicative of moderate stress. Including introduction to the task, subjects were exposed to the stressful situation for a total of 15 min. Thereafter, participants filled in the mood scale a second time, followed by six visual analog scales. Additional blood and saliva samples were obtained 10, 20, 30, 45, 60, and 90 min after the cessation of stress. Finally, the subjects were asked to guess whether they had received an estradiol or a placebo patch and whether they had experienced any psychological or physical symptoms after the patch was applied.

Blood and saliva sampling

Ethylenediamine tetraacetate blood samples were immediately put in an ice bath, and saliva samples were stored at room temperature until completion of the session. Saliva was collected by the subjects using Salivette (Sarstedt, Rommelsdorf, Germany) collection devices. They gently chewed for 20–60 s until the cotton roll was soaked with saliva. The devices were stored at −20 C until biochemical analysis. Before assaying the samples for cortisol, they were thawed and spun at 3000 rpm for 10 min. This procedure usually results in 0.5–1 mL low viscosity saliva.

Biochemical analyses

Estradiol and testosterone levels were measured in the −1 min samples employing a commercial enzyme-linked immunosorbent assay kit (IBL, Hamburg, Germany). ACTH was measured with a commercial two-site chemiluminescence assay (Nichols Institute, Bad Manheim, Germany). The free cortisol concentration in saliva was determined using a time-resolved immunoassay with fluorometric detection, as described in detail previously (16). Inter- and intraassay coefficients of variance were below 10% for all analytes. Norepinephrine was assayed by high performance liquid chromatography with electrochemical detection, as described by Smedes *et al.* (17). The intraassay coefficient of variance at 258 pg/mL was 3.4% (n = 8) for this method.

Heart rate

Heart rates were measured continuously at 1-min intervals with EKC precision employing wireless transmission (Sport Tester Profi, Polar Instruments, Grob-Gerau, Germany). From −10 to 25 min relative to stress onset, values were collapsed to 5-min blocks. Heart rate responses were computed from the five poststress 5-min blocks minus the individual mean heart rates at rest (−5 to −1 min).

Psychological assessment

For mood ratings before and after stress, a German questionnaire was employed that is especially suited for repeated measures within several minutes to hours (Beschwerdeliste BSKE-ak) (18). The 28 items are rated on a 7-point scale ranging from 0 = not at all to 6 = very strongly. Factor analyses revealed 4 scales that the authors of the questionnaire termed positive emotionality, negative emotionality, aggressiveness, and anxiety.

Six visual analogue scales (VAS) were employed for subjective ratings of the stressfulness of the free speech/arithmetics task. The following items were used: stressful, uncontrollable, novel, unpredictable, ego involvement, and anticipation of negative consequences. The ratings were transformed into values between 0 = not at all to 100 = absolutely.

Statistical analyses

A χ^2 analysis was used to test whether subjects assigned themselves correctly to the estradiol or placebo group and whether volunteers with the estradiol patch experienced more psychological and/or bodily changes than the controls.

Student's *t* tests were employed for comparisons of estradiol levels (one-tailed) as well as for comparing age, body mass index, mood, and VAS stress ratings (two-tailed) between the two treatment groups. Bonferroni corrections were applied for assessing possible pre/post differences in mood (four comparisons; $\alpha = 0.013$), group differences in mood (eight comparisons; $\alpha = 0.006$), and VAS stress rating (six comparisons; $\alpha = 0.008$).

ANOVAs for repeated measures were computed to reveal time and group by time effects for ACTH, cortisol, norepinephrine, and heart rate in response to the stressful procedure. All reported results were corrected by the Greenhouse/Geisser procedure (reflected by the degrees of freedom with decimal values). For significant effects, Newman-Keuls *post-hoc* tests were applied. Pearson correlations were computed for the areas under the individual response curves (trapezoid formula; one-tailed). Significant effects were assumed at $\alpha = 0.05$. All results shown are the mean ± SE.

Results

As summarized in Table 1, the estradiol group did not differ from the placebo group in age or body mass index; however, they had significantly higher estradiol and lower testosterone levels than the control subjects (estradiol: $t_{31} = 3.03$; $P = 0.002$; testosterone: $t_{31} = 2.29$; $P = 0.015$). Subjects who received an estradiol patch were unable to correctly identify the nature of the patch, and the same number of subjects experienced side-effects of the patch in both groups (Table 2).

The 15-min stress exposure led to significant endocrine, cardiovascular, as well as psychological responses. In the total group, the levels of ACTH, cortisol, and norepinephrine showed significant increases, with 1.5- to 2-fold changes from baseline values. ACTH and norepinephrine concentrations peaked 10 min after cessation of the stressor with steadily decreasing values thereafter (time effect, ACTH: $F_{2.3,64.7} = 20.67$; $P < 0.0001$; norepinephrine: $F_{2.8,82.0} = 9.27$; $P < 0.0001$). As expected, cortisol levels peaked, with a 10-min time lag compared to the peak for ACTH, 20 min after stress (time effect: $F_{2.6,77.6} = 41.78$; $P < 0.0001$). Heart rates were highest 6–15 min after stress onset (time effect: $F_{2.6,71.3} = 17.38$; $P < 0.0001$).

Although the response kinetics were similar, the two groups differed significantly in their endocrine response magnitudes. As depicted in Fig. 1, A and B, the estradiol group showed exaggerated ACTH and cortisol responses to the stress (group by time, ACTH: $F_{2.3,64.7} = 3.41$; $P < 0.05$; cortisol: $F_{2.6,77.6} = 4.61$, $P = 0.005$). Although ACTH baselines appeared to be elevated in the estradiol group, *post-hoc* analyses did not support this idea. However, subjects who received estradiol treatment had significantly higher ACTH levels 10 and 20 min poststress (both $P < 0.001$) as well as higher cortisol levels at 10 min ($P = 0.0002$), 20 and 30 min ($P < 0.0001$), and 45 min poststress ($P = 0.03$), respectively.

TABLE 1. Estradiol, age, and body mass index (BMI) in the two experimental groups

	Estradiol group	Placebo group	P
Estradiol (pg/ml)	61.9 ± 5.2	38.5 ± 5.7	0.002
Testosterone (ng/ml)	5.63 ± 0.4	6.80 ± 0.3	0.015
Age (yr)	24.6 ± 1.0	23.9 ± 0.7	NS
BMI	22.5 ± 0.4	22.8 ± 0.4	NS

Values are the mean ± SE.

TABLE 2. Number of subjects who "guessed" that they had received a verum *vs.* placebo patch and the number of side-effects they experienced

	Patch "guess"		Side-effects
	Placebo	Estradiol	
Estradiol group	12	4	3
Placebo group	13	3	3

Although norepinephrine levels followed the same pattern, the differences in response magnitude between the two groups did not differ statistically when compared by ANOVA (Fig. 1C). However, the area under the response curve (trapezoid formula) was greater for the estradiol group (530 ± 126 *vs.* 162 ± 92; $t_{29} = 2.39$; $P = 0.024$). No statistically significant group difference was observed in the heart rate response (Fig. 2). Again, men treated with estradiol tended to show higher heart rate responses during the first 15 min of stress. Cortisol responses were significantly correlated with ACTH and heart rate changes ($r = 0.62$; $P < 0.001$ and $r = 0.50$; $P = 0.002$, respectively). Also, norepinephrine and heart rate responses were positively associated ($r = 0.35$; $P = 0.035$).

As for psychological responses, there was a significant increase in positive emotionality ($t_{31} = 3.71$; $P = 0.0008$) and aggressiveness ($t_{31} = 3.59$; $P = 0.001$), with no changes in negative emotionality or anxiety in response to the stressful procedure in the total group. As shown in Table 3, estradiol and placebo subjects reported comparable moods both before and after the experiment. Also, they rated the stress similar with respect to the six items of the VAS after Bonferroni correction (α-level was 0.008 for the six items). There were no significant correlations between endocrine and heart rate changes and mood or VAS ratings.

Discussion

The present findings suggest that short term treatment with estradiol leads to enhanced hypothalamic-pituitary-adrenal (HPA) and sympathetic responsiveness to psychological stress in healthy men. This change was neither accompanied by side-effects of the medication nor by differences in mood or subjective stress ratings. Although ACTH levels in the estradiol group followed the time course of the control group (on a higher level, however), the cortisol time course appeared to be altered in the estradiol-treated volunteers. Their cortisol concentrations continued to rise for another 10 min until they reached a peak 20 min after the cessation of stress. This is rather unusual because in numerous studies we observed that our stress protocol induces peak salivary cortisol levels 10 min after completion. The prolonged increase in the estradiol group is in accord with findings by Burgess and Handa (10), who reported that corticosterone levels rose over a prolonged time in ovariectomized rats treated with estradiol. Along with data from *in vitro* receptor studies, the researchers concluded that estradiol may cause an impairment of the glucocorticoid-receptor mediated slow negative feedback. This appears to be a possible explanation for the observed cortisol effect in the present study, too. The estradiol-treated men showed a mean cortisol response compa-

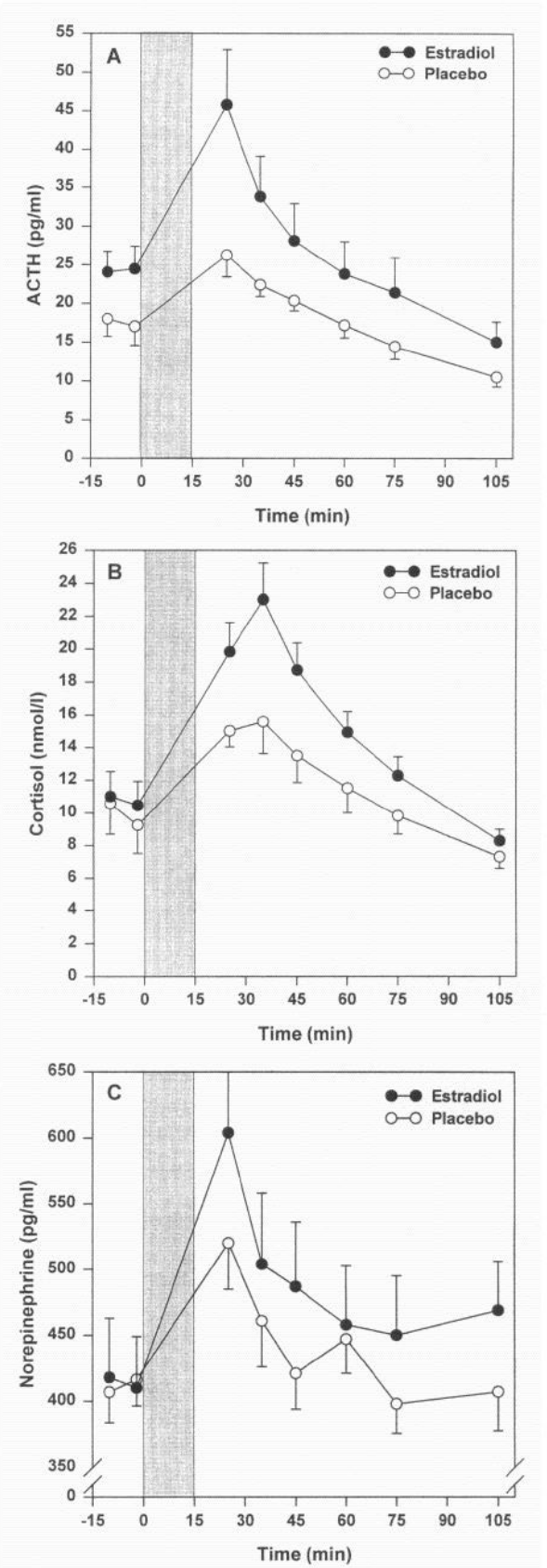

FIG. 1. Mean ACTH (A), saliva cortisol (B), and norepinephrine (C) levels (±SE) before and after stress (Trier Social Stress Test). The *shaded area* indicates the period of stress exposure.

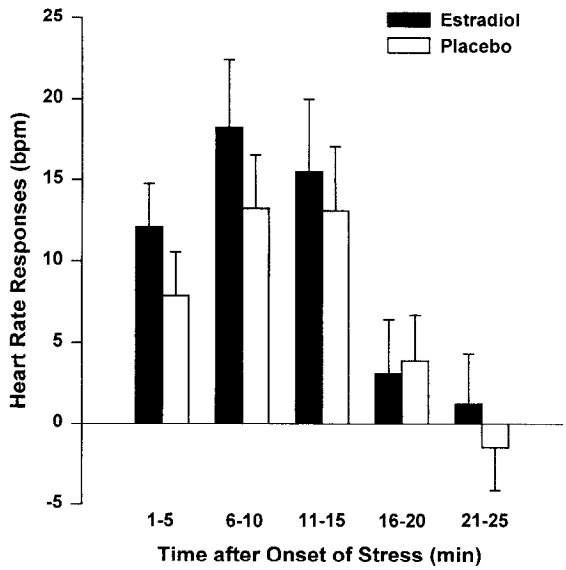

FIG. 2. Heart rate changes (±SE) in response to stress (Trier Social Stress Test).

TABLE 3. Mood ratings (before and after stress, TSST) and subjective VAS stress ratings of the TSST procedure

	Placebo	Estradiol	P
Mood rating			
Positive emotionality pre	18.00 ± 4.2	17.56 ± 3.2	NS
Positive emotionality post	20.63 ± 5.0	21.53 ± 5.1	NS
Negative emotionality pre	10.13 ± 7.4	9.50 ± 5.2	NS
Negative emotionality post	8.88 ± 6.3	7.87 ± 5.6	NS
Agressiveness pre	5.25 ± 4.7	4.88 ± 3.6	NS
Agressiveness post	7.30 ± 5.6	9.00 ± 5.2	NS
Anxiety pre	2.88 ± 2.6	2.44 ± 1.9	NS
Anxiety post	2.44 ± 2.0	2.07 ± 2.2	NS
Stress rating			
Stressfulness	33.69 ± 19.5	48.75 ± 15.6	NS
Uncontrollability	26.56 ± 25.8	24.69 ± 21.3	NS
Novelty	36.94 ± 28.1	52.50 ± 29.3	NS
Unpredictability	41.50 ± 26.6	42.44 ± 21.5	NS
Ego Involvement	22.50 ± 23.3	34.75 ± 21.8	NS
Anticipation of negative consequences	14.75 ± 20.8	15.71 ± 15.2	NS

Values are the mean ± SD.

rable in magnitude to that of a small subgroup of high responders observed recently in our laboratory (20).

The observed estradiol treatment-induced decrease in testosterone levels supports similar findings reported recently (21, 22). Estradiol exerts negative feedback action on hypothalamic and pituitary sites, which leads to a decreased release of GnRH and LH. It is unlikely that the small decrease in testosterone levels observed here (~15%) caused the enhanced ACTH and cortisol responsiveness, because early studies by Kitay (7) showed that testosterone depressed ACTH secretion and steroid clearance in rats.

Although data from animal studies are in agreement with the results of the present study (6–13, 23), two other experiments with human subjects obtained seemingly conflicting results. Compared with placebo-treated women, Lindheim and co-workers (24) found blunted ACTH and cortisol responses to a mental stressor in postmenopausal women after estradiol treatment for 6 weeks. However, these women were tested with the identical mental stressor twice, before and after the pharmacological treatment. As HPA responses to stress appear to habituate readily upon repeated stress exposure in the majority of subjects (20), the confoundation of habituation and estradiol application makes it difficult to ascribe the observed effects to the pharmacological treatment.

Our findings of enhanced norepinephrine responses (with the same, nonsignificant trend for heart rate) in the estradiol group contradicts data reported by Del Rio *et al.* (25). This is somewhat surprising, as they, too, studied healthy men and applied an identical estradiol dose over the same period that we did. However, their experimental protocol differed in several aspects from the one used in the present study. First, Del Rio *et al.* employed a stressor that elicited only minor cardiovascular responses. Mean heart rate increases were 2–6 beats/min in the placebo and estradiol sessions, respectively, and norepinephrine levels did not change significantly from baseline values. In contrast, we obtained mean peak heart rate increases between 14–18 beats/min and a highly significant stress effect for norepinephrine levels, indicating a much greater sympathetic response. Moreover, the subjects in the Del Rio experiment were exposed to the same stressor twice, which probably produced a larger error variance, again due to the confoundation of stressor and experience (or habituation).

Which mechanisms were responsible for an enhanced HPA responsiveness and a greater sympathetic output after short term treatment with estradiol? There is evidence for a functional change in mineralocorticoid and glucocorticoid receptors in various brain regions, including the hippocampus (10, 12, 14, 26, 27), hypothalamus, and amygdala (26), induced by estradiol in rats. However, we doubt that a 24- to 48-h moderate elevation of estradiol levels in men could yield such a profound central effect. Alternatively, increased synthesis of vasopressin and subsequent corelease with CRH from hypothalamic neurons could probably explain the stimulatory effect of estradiol. Using the same transdermal estradiol patch as that employed in the present study, Bossmar and colleagues (19) recently reported that a 5-day treatment with estradiol significantly increased plasma vasopressin levels. If a short term estradiol treatment would also enhance coexpression and secretion of vasopressin in CRH paraventricular neurons of the hypothalamus, the HPA axis would become hyperresponsive and possibly explain our findings in healthy men. On the other hand, a chronically elevated estradiol level could initially lead to an increased receptor/ligand availability, but eventually would produce a downregulation of the system and thus cause reduced HPA activity. This was recently shown for vasopressinergic receptors observed in women using oral contraceptives (19) and CRH immunoreactivity in the median eminence of rats (28). Taken together, these results suggest that estradiol affects the major sites responsible for HPA regulation in the central nervous system. It could, therefore, be of interest to investigate potential HPA response differences to stimuli acting at the level of the central nervous system (*e.g.* psychosocial stress) after short and long term estradiol treatment and compare these responses to endocrine changes after the

injection of CRH and ACTH in the same subjects. These questions are being currently studied in our laboratory.

Finally, the results of increased endocrine responses in estradiol-treated men without parallel changes in subjective stress experience suggest that pituitary-adrenal responses are inaccurate measures of stress. Although stress of increasing intensity will lead to overall increases in ACTH and cortisol levels, we frequently observed that subjects are often not able (or willing) to report the respective changes in mood or well-being. Especially in male volunteers, introspective stress ratings rarely correlate with physiological stress indexes.

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