

# Dose-dependent effects of 17- $\beta$ -estradiol on pituitary thyrotropin content and secretion *in vitro*

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## Abstract

We studied the basal and thyrotropin-releasing hormone (TRH) (50 nM) induced thyrotropin (TSH) release in isolated hemipituitaries of ovariectomized rats treated with near-physiological or high doses of 17- $\beta$ -estradiol benzoate (EB; *sc*, daily for 10 days) or with vehicle (untreated control rats, OVX). One group was sham-operated (normal control). The anterior pituitary glands were incubated in Krebs-Ringer bicarbonate medium, pH 7.4, at 37°C in an atmosphere of 95% O<sub>2</sub>/5% CO<sub>2</sub>. Medium and pituitary TSH was measured by specific RIA (NIDDK-RP-3). Ovariectomy induced a decrease ( $P < 0.05$ ) in basal TSH release (normal control =  $44.1 \pm 7.2$ ; OVX =  $14.7 \pm 3.0$  ng/ml) and tended to reduce TRH-stimulated TSH release (normal control =  $33.0 \pm 8.1$ ; OVX =  $16.6 \pm 2.4$  ng/ml). The lowest dose of EB (0.7  $\mu$ g/100 g body weight) did not reverse this alteration, but markedly increased the pituitary TSH content ( $0.6 \pm 0.06$   $\mu$ g/hemipituitary;  $P < 0.05$ ) above that of OVX ( $0.4 \pm 0.03$   $\mu$ g/hemipituitary) and normal rats ( $0.46 \pm 0.03$   $\mu$ g/hemipituitary). The intermediate EB dose (1.4  $\mu$ g/100 g body weight) induced a nonsignificant tendency to a higher TSH response to TRH compared to OVX and a lower response compared to normal rats. Conversely, in the rats treated with the highest dose (14  $\mu$ g/100 g body weight), serum 17- $\beta$ -estradiol was 17 times higher than normal, and the basal and TRH-stimulated TSH release, as well as the pituitary TSH content, was significantly ( $P < 0.05$ ) reduced compared to normal rats and tended to be even lower than the values observed for the vehicle-treated OVX group, suggesting an inhibitory effect of hyperestrogenism. In conclusion, while reinforcing the concept of a positive physiological regulatory role of estradiol on the TSH response to TRH and on the pituitary stores of the hormone, the present results suggest an inhibitory effect of high levels of estrogen on these responses.

## Key words

- Thyrotropin (TSH)
- Ovariectomized rats
- 17- $\beta$ -estradiol benzoate
- Thyrotropin-releasing hormone (TRH)

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## Introduction

The role of estrogen in the regulation of thyrotropin (TSH) secretion is not understood. Ovariectomy of adult female rats may cause a reduction (1) or no change (2-4) in plasma and pituitary TSH concentrations.

Estradiol administered to ovariectomized (OVX) rats has been shown to increase serum and pituitary TSH (1,3). Several studies suggest that estrogen stimulates the TSH response to thyrotropin-releasing hormone (TRH) both in rats (1) and humans (5,6). It has been shown that estradiol positively regu-

lates the number of TRH receptors (1) and TRH receptor gene expression (7). On the other hand, negative effects of estradiol have also been reported. In normal female rats, estradiol treatment decreased the pituitary hormonal content (8-10) without changing serum TSH (8,9). It was also reported that estradiol augments the suppressive effects of thyroid hormones on TSH synthesis (11,12) and secretion (12). Early studies (13,14) in which bioassays were employed to quantify TSH have suggested that estradiol has a biphasic effect on plasma and pituitary TSH, being stimulatory at lower doses and inhibitory at higher doses. In more recent years, as reviewed previously (1-12), both positive and negative modulatory effects of estrogen have been reported. However, the studies did not focus on dose-related effects, a fact that may partially explain the conflicting results. Therefore, in the present study we treated ovariectomized rats with 17- $\beta$ -estradiol benzoate (EB) at doses that would raise serum estradiol to near-physiological or to high concentrations in order to examine the physiological role of estrogen and the effects of hyperestrogenism on basal and TRH-induced TSH secretion and on pituitary TSH content.

## Material and Methods

### Animal treatment

Adult female rats (150-250 g) bred in our animal facilities were housed under controlled conditions of temperature ( $24 \pm 1^\circ\text{C}$ ) and light (12 h light starting at 7:00 a.m.). All rats showed regular 4-5 days estrous cycles monitored by vaginal cytology for at least two weeks before starting the experiments. Groups of animals were ovariectomized and others were subjected to surgical stress (sham-operated normal) and used as controls. In separate experiments, OVX rats were treated with 0.7 (experiment 1) or 1.4 or 14 (experiment 2)  $\mu\text{g}$  estradiol benzoate/100 g body

weight (Sigma Chemical Co., St. Louis, MO) in 0.2 ml corn oil injected *sc* daily for 10 days. Rats were sacrificed 24 h after the last injection and three weeks after ovariectomy. One group of ovariectomized rats was used as estradiol controls and they received vehicle instead of EB. The sham-operated normal group also received vehicle rather than estradiol.

### Basal and TRH-stimulated TSH release *in vitro*

After the treatment period described above, the animals were sacrificed by decapitation, trunk blood was collected, and serum was separated and stored at  $-20^\circ\text{C}$  until the time for assay. The pituitaries were quickly dissected out, the anterior pituitary was separated from the posterior pituitary and transected with a longitudinal midline cut. Each anterior hemipituitary was immediately transferred to a tube containing 1 ml of Krebs-Ringer bicarbonate medium, pH 7.4, and incubated at  $37^\circ\text{C}$  in an atmosphere of 95%  $\text{O}_2$ /5%  $\text{CO}_2$  in a Dubnoff metabolic shaker (50 cycles/min). After a 30-min preincubation period, the medium was removed and the hemipituitaries were resuspended in 1 ml of fresh medium. After 1-h incubation an aliquot was removed for measurement of basal TSH, and TRH (Sigma) was then added to a final concentration of 50 nM in all groups. The pituitaries were incubated for an additional 30 min to determine TSH release in response to TRH. Each hemipituitary was homogenized in phosphate-buffered saline, pH 7.6, for measurement of intrapituitary TSH content.

### Hormone measurements

TSH was measured by radioimmunoassay with kits supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD) and reported in terms of the reference preparation (RP2).

17- $\beta$ -Estradiol was measured by radioimmunoassay (Kit Coat A Count, Diagnostic Products Corporation, Los Angeles, CA) in the serum of ovariectomized rats treated with EB and in the serum of normal adult female rats matched for weight and age to the rats used in the experiments described above, during different phases of the estrous cycle.

### Statistical analysis

Data are reported as means  $\pm$  SEM. One-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls multiple comparison test was employed to assess the significance of all data except for serum TSH, which was analyzed by the Kruskal-Wallis test followed by the Dunn comparison test.  $P < 0.05$  was considered to be significant.

### Results

As shown in Table 1, rats treated daily with 0.7 and 1.4  $\mu$ g 17- $\beta$ -estradiol benzoate/100 g body weight for 10 days showed serum estradiol concentrations higher than those of normal rats but much closer to physiological levels than those found in rats receiving 14  $\mu$ g/100 g body weight. The rats receiving 14  $\mu$ g/100 g body weight were highly hyperestrogenized, since serum estradiol exceeded the proestrus concentration by a factor of almost 17. We have previously reported that in our *in vitro* system, the basal and TRH-stimulated TSH release was not significantly different among pituitaries from rats in the various estrous phases, although we found a slight trend to higher values of basal TSH release during proestrus (15). The rats of the sham-operated group (normal) in this study were all in diestrus, except one that was in estrus. Confirming the near-physiological estradiol status of the animals treated with the lower EB doses, the reduced uterine weight of castrated rats was almost completely recovered compared to the normal group at 0.7  $\mu$ g/100 g body weight (3.4 times

higher than OVX but 15% lower than normal,  $P < 0.05$  vs normal and OVX) and fully recovered by the treatment with 1.4  $\mu$ g EB/100 g body weight. In the hyperestrogenized rats, the uterine weight was higher (35%) than that of the normal group. Neither the ovariectomy nor the estrogen treatments significantly changed the body weight of the rats (data not shown).

Serum TSH was not significantly affected by ovariectomy or estrogen treatment (Table 1).

The hemipituitaries from ovariectomized rats showed a significant ( $P < 0.05$ ) reduction in basal TSH release (Figure 1A), and a non-significant trend to lower TRH-stimulated TSH release (Figure 1B). The intrapituitary content of TSH was not significantly reduced by ovariectomy (Figure 1C). The treatment of OVX rats with 0.7  $\mu$ g EB/100 g body weight for 10 days had no significant effect on the reduction of *in vitro* basal TSH release caused by ovariectomy (Figure 1A), and also did not significantly change the TRH-stimulated TSH release (Figure 1B). However, the pituitary TSH content was significantly ( $P < 0.05$ ) increased in the EB-treated group compared to both the OVX and the normal sham-operated group (Figure 1C).

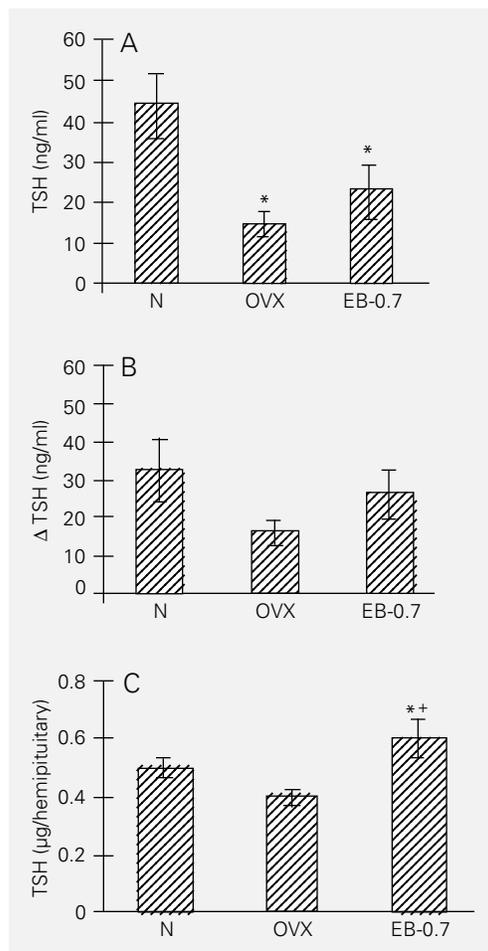
Basal TSH release from hemipituitaries

Table 1 - Serum estradiol and serum TSH of normal female rats during the estrous cycle and of vehicle (OVX)- or estradiol benzoate (EB)-treated ovariectomized rats.

\* $P < 0.05$  vs normal rats (one-way ANOVA followed by Student-Newman-Keuls test for serum estradiol and Kruskal-Wallis followed by Dunn test for serum TSH).

Groups	Serum estradiol (pg/ml)	Serum TSH (ng/ml)
Normal rats		
Proestrus	61.2 $\pm$ 2.7	0.7 $\pm$ 0.10
Estrus	38.2 $\pm$ 4.2	0.6 $\pm$ 0.10
Diestrus I	48.6 $\pm$ 6.9	0.6 $\pm$ 0.10
Diestrus II	45.0 $\pm$ 5.5	0.5 $\pm$ 0.10
Ovariectomized rats		
OVX	-	0.6 $\pm$ 0.50
OVX + EB 0.7 $\mu$ g/100 g body weight	90.4 $\pm$ 12.7*	0.6 $\pm$ 0.04
OVX + EB 1.4 $\mu$ g/100 g body weight	177.0 $\pm$ 13.0*	0.7 $\pm$ 0.04
OVX + EB 14 $\mu$ g/100 g body weight	1031.0 $\pm$ 254*	0.8 $\pm$ 0.04

Figure 1 - Effect of estradiol on TSH release. TSH release from incubated hemipituitaries was measured for sham-ovariectomized rats treated with vehicle (N) and ovariectomized rats treated with vehicle (OVX) or estradiol benzoate (EB-0.7; 0.7  $\mu\text{g}$  EB/100 g body weight, sc, daily for 10 days). *Panel A*, Basal release; *panel B*, TRH-stimulated TSH release;  $\Delta$  TSH = difference between medium TSH after TRH and basal TSH; *panel C*, intrapituitary TSH of the incubated glands. Data are reported as means  $\pm$  SEM for 9-11 hemipituitaries per group. \* $P < 0.05$  vs the normal group and + $P < 0.05$  vs the OVX group (one-way ANOVA followed by Student-Newman-Keuls test for comparison among the groups).



of OVX rats treated with a high dose of EB (14  $\mu\text{g}/100$  g body weight) was significantly ( $P < 0.05$ ) decreased compared to normal rats, but was not different from the OVX group or the OVX group treated with 1.4  $\mu\text{g}$  EB/100 g body weight (Figure 2A). Regarding the TSH response to TRH, the glands from OVX rats treated with 14  $\mu\text{g}$  EB/100 g body weight showed a significantly decreased response ( $P < 0.05$ ) compared to the normal group, but not compared to the OVX group. The OVX group receiving 1.4  $\mu\text{g}$  EB/100 g body weight presented only a nonsignificant trend to increased TRH-stimulated TSH release compared to the OVX group (Figure 2B). As shown in Figure 2C, the intrapituitary TSH from the OVX group receiving 14  $\mu\text{g}$  EB/100 g body weight was decreased compared to normal and to OVX rats treated or not

with 1.4  $\mu\text{g}$  EB/100 g body weight ( $P < 0.05$ ).

The hemipituitaries from ovariectomized rats showed a reduction in basal ( $P < 0.05$ ) and TRH-induced TSH release compared to the glands of normal animals (Figure 1A,B). Despite the decreased TSH release in response to TRH, the incubated pituitaries from the ovariectomized rats showed a nonsignificant trend to lower TSH content (Figures 1C and 2C).

## Discussion

The results presented here provide evidence that administration of estradiol benzoate to ovariectomized rats at the doses of 0.7 and 1.4  $\mu\text{g}/100$  g body weight, daily for 10 days, increases pituitary TSH content and tends to increase responsiveness to TRH. However, we report here for the first time that at high doses which induced hyperestrogenism, estradiol had the opposite effect, significantly decreasing the intrapituitary TSH content and probably also the thyrotrope responsiveness to TRH. The latter possibility is suggested by the fact that the reduction in TRH response was greater than that induced by castration alone.

Other authors have shown that estradiol administration to ovariectomized rats recovered the reduced TSH response to TRH of the castrated animals (1,16). However, this stimulatory effect was not found by Christianson et al. (2), even though they employed similar doses of EB (0.33  $\mu\text{g}/100$  g body weight for 10 days and 0.25  $\mu\text{g}/100$  g body weight for 7 days). Chen and Walfish (3,4), employing a dose similar to the intermediary EB dose of the present study (2  $\mu\text{g}/100$  g body weight for 7 days), also found a stimulatory effect of estrogen on the TSH response to TRH. Therefore, our *in vitro* data are in agreement with those obtained in *in vivo* studies (1,16) and support the concept that estradiol is physiologically important to maintain the normal response to TRH in females. The induction of increasing num-

bers of TRH receptors in the pituitary gland (1) seems to be the mechanism by which estrogen stimulates the TSH response to TRH. Recently, Kimura et al. (7) demonstrated that estradiol increases the synthesis of TRH receptors of pituitary cells from normal rats and GH<sub>3</sub> cells by increasing the transcription rate and stability of the TRH receptor mRNA. Therefore, our finding of a decreased response to TRH in rats treated with the largest dose of EB suggests that in hyperestrogenism there are other direct or indirect estrogen effects in addition to the TRH receptor level.

Estradiol seems to modulate the pituitary TSH stores in a dose-related manner. The treatment with the lowest EB dose (0.7 µg/100 g body weight) significantly stimulated the intrapituitary TSH pool in the ovariectomized rats. Conversely, in the highly hyperestrogenized rats, pituitary TSH content was decreased. These findings suggest that, physiologically, estrogen stimulates while excess of the steroid causes depletion of TSH stores. Since serum TSH was not affected significantly either by ovariectomy or by estrogen treatment, it is possible that the changes in pituitary TSH content were due to a relative decrease in the rate of hormone synthesis. This possibility could be tested experimentally. Estradiol treatment of normal female rats had no influence on TSH β mRNA abundance (11,12), but was able to reduce the effect of hypothyroidism (11,12) and to amplify the T<sub>3</sub>-induced suppression of TSH β mRNA in hypothyroid rats (12). These findings suggest that estradiol might have a negative influence on β TSH gene expression. However, it is not known if this effect is dose dependent.

Hyperestrogenism present in pregnancy causes some changes in pituitary-thyroid axis function. Early in pregnancy, increased renal iodine clearance leads to increased thyroidal iodine uptake and has, therefore, little impact on the thyroid function of normal pregnant women from iodine-sufficient areas (17). However, in the first trimester preg-

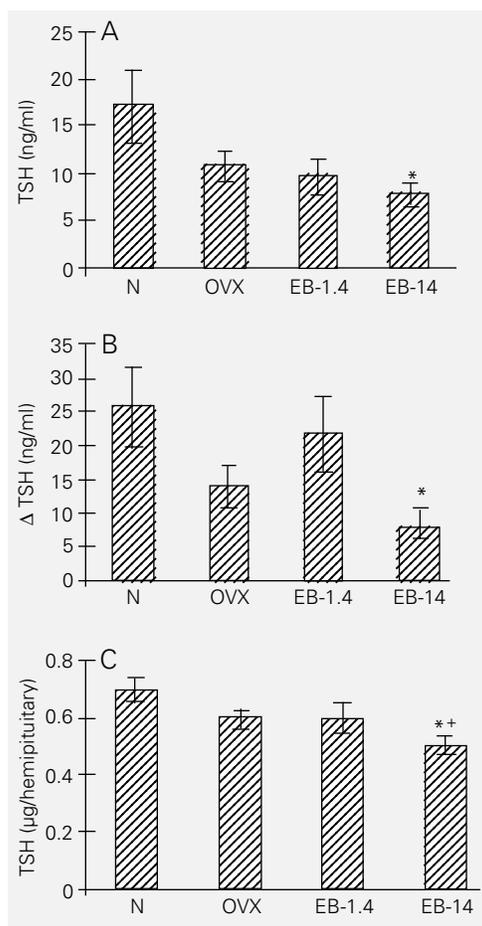


Figure 2 - Effect of estradiol concentration on TSH release. TSH release from incubated hemipituitaries was measured for sham-operated (N) or ovariectomized rats treated with vehicle (OVX) or 1.4 (EB-1.4) or 14 (EB-14) µg estradiol benzoate/100 g body weight (sc, daily for 10 days). *Panel A*, Basal release; *panel B*, TRH-stimulated TSH release; Δ TSH = difference between medium TSH after TRH and basal TSH; *panel C*, intrapituitary TSH of the incubated glands. Data are reported as means ± SEM for 8-11 hemipituitaries per group. \*P < 0.05 vs the normal group and +P < 0.05 vs the OVX groups (one-way ANOVA followed by Student-Newman-Keuls test for comparison among the groups).

nant women show a small transitory increase in free thyroxine, a decrease in thyrotropin, and a decrease in the TSH response to TRH (17). These alterations have been attributed to hyperestrogenism which causes an increment in serum concentration of thyroxine-binding globulin together with the first-trimester increase in human chorionic gonadotropin (17). However, it is not known if there is a direct effect of the high serum estrogen on the pituitary gland of pregnant women. Our data are probably not related to estrogen actions on thyroid hormone carrier proteins because it was shown (18) that estrogen did not change the serum thyroxine-binding capacity of rats, although it decreased the binding capacity of transthyretin. Furthermore, it has been shown experimentally in rats that estradiol has direct effects on the pituitary gland (13,14), changing TSH secretion.

It may be argued that the lack of other ovarian hormones, mainly progesterone, might have some influence on the results obtained here. However, Watanobe and Takebe (1) reported that progesterone treatment of ovariectomized rats had no effect on serum or pituitary TSH content or on the response to TRH.

We thus confirmed the earlier observations of Fisher and D'Angelo (14) who suggested a biphasic effect of estrogen on TSH content. However, contrary to those studies we were not able to find significant changes in serum TSH. This may be related to the differences in experimental design and methods. Those authors used bioassays to evaluate TSH, they did not measure serum estradiol and they employed normal rats instead of castrated ones, as we did in the present

study. The fact that serum TSH was not significantly changed by the different treatments suggests that *in vivo* adjustments were acting in order to compensate for the alterations in TRH responsiveness and TSH content in the pituitary gland.

Thus, estradiol at high but close to physiological serum concentrations had a stimulatory effect on pituitary TSH content and TRH-stimulated TSH secretion *in vitro*. Conversely, hyperestrogenism seems to depress the ability of the pituitaries from ovariectomized rats to respond to TRH and induced a depletion in their TSH stores.

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