Effects of oestradiol benzoate on the pituitary secretion and peripheral kinetics of thyrotrophin in the rat

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Abstract. Previous works from this laboratory have demonstrated that oestradiol benzoate (EB) in euthyroid male and female rats induced a significant decrease in the pituitary content of TSH while serum levels of this hormone remained normal. The present work studied the effects of EB (25 µg/100 g body weight, during 9 days) on the peripheral metabolism of [125I]rTSH and on the pituitary and plasma concentration of TSH in euthyroid and hypothyroid rats. No significant variations were observed in [125I]rTSH kinetics of EB-treated euthyroid rats vs untreated controls: fractional turnover rate 2.8 ± 0.2 vs 3.0 ± 0.3%/min, distribution space 6.5 ± 0.4 vs 6.8 ± 0.5 ml/100 g body weight, disposal rate 18.4 ± 2.4 vs 18.1 ± 1.9 µU/100 g/min and extrapituitary pool 645 ± 42 vs 614 ± 43 µU/100 g body weight. Similarly, in hypothyroid rats oestrogens induced no changes in TSH kinetics except for an increase in distribution space (P < 0.025). However, oestrogens decreased the pituitary pool of TSH (P < 0.001) in both euthyroid and hypothyroid rats and increased the plasma TSH in hypothyroid animals (P < 0.01), all vs their respective controls. Neither hypothyroid group had detectable plasma levels of T4 and T3. In summary: 1) the marked decrease of pituitary TSH with normal plasma TSH induced by EB appears unrelated to the peripheral metabolism of TSH, 2) the results from hypothyroid rats suggest that EB stimulates the release of TSH from the pituitary gland.

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Material and Methods

Wistar rats weighing approximately 200 g were maintained on Purina rat chow and water ad libitum. The animals were injected daily with 25 µg of EB (Schering, Argentina)/100 g body weight in 100 µl of sunflower oil, sc, during 9 days. Control rats were injected with the vehicle alone. Hypothyroidism was induced by the administration of 131I, ip. The animals were studied 3 months later when serum levels of T4 and T3 were not detectable.

[125I]rTSH kinetics

The animals received and intracardiac injection of [125I]rTSH (106 CPM, specific activity 80 µCi/µg,
NIAMDD-rat TSH-I-4) in 100 µl of normal saline, using a butterfly needle. Two hundred and fifty µl heparinized blood samples, replaced by an equal volume of normal saline, were obtained at 0, 10, 20, 40 and 80 min after the injection of labelled TSH. All samples were drawn by cardiac puncture with individual butterfly needles for each extraction, under light ether anaesthesia. After the last sample was drawn the animals were killed, the hypophyses removed immediately and homogenized in PBS buffer as reported elsewhere (Boado et al. 1983). Plasma [125I]-TSH was measured by immunoprecipitation. Duplicate aliquots of 50 µl of plasma and standard injected were incubated with 100 µl NIAMDD-anti-rat TSH serum-5 (1:500 and 1:1000) for hypothyroid and euthyroid samples, respectively, PBS buffer, 0.05 M EDTA, 1% normal rabbit serum) for 24 h at room temperature. Thereafter, the second antibody was added, incubated 20 h at 4°C and centrifuged. The immunoprecipitate was counted in a well-type detector (Picker, Compac 120). The peripheral metabolism of [125I]-TSH was assessed by methods described elsewhere for T4 peripheral kinetics (Zaninovich et al. 1977) using the semilogarithmic regression slope of plasma [125I]-TSH as determined by the method of least squares. Disposal rate of TSH was obtained by the product of fractional turnover rate and extrapituitary pool. TSH was labelled with 125I (New England Nuclear) using the stoichiometric chloramine T method (Boado et al. 1982a).

**TRH test**

Heparinized blood samples were drawn immediately before and 10 min after the iv injection of 1 µg TRH (Hoffman LaRoche, Basel)/100 g body weight. This dose and timing of TRH test provided maximum response in this and other laboratories (Lifschitz et al. 1978; Boado et al. 1982b).

**RIAs**

Plasma and pituitary TSH were measured using the protocol and reagents provided by the National Pituitary Agency (NIAMDD, USA) with slight modifications (Silva & Larsen 1978; Boado et al. 1982a). TSH was labelled as described above. Plasma T4 and T3 were determined using a double antibody technique (Chopra 1972; Boado et al., in press). [125I]-labelled T4 and T3 were obtained from New England Nuclear with a specific activity of approximately 1500 µCi/µg. The antisera were purchased from Radioassay System Laboratory (USA). The Pall-M 4-logistic analysis method (Picker, Compac 120) was used to calculate hormone concentration using 5% as maximal standard error. All samples were measured in the same RIA. The limit of detection was 0.8 µU/tube, 0.4 µg/dl and 10 ng/dl for TSH, T4 and T3, respectively.

Proteins were measured by the method of Lowry et al. (1951) using bovine serum albumin as standard (Sigma Chemical). Probability value was obtained by Student's t-test.

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**Table 1. Effects of EB on the [125I]-TSH peripheral kinetics in euthyroid and hypothyroid female rats.**

<table>
<thead>
<tr>
<th></th>
<th>Euthyroid rats</th>
<th>Hypothyroid rats</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control (6)¹</td>
<td>EB-treated (6)</td>
</tr>
<tr>
<td>Fractional turnover</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rate (%/min)</td>
<td>3.0 ± 0.3</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Distribution space</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ml/100 g body weight)</td>
<td>6.8 ± 0.5</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>MCR (ml/100 g/min)</td>
<td>0.20 ± 0.03</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>Plasma TSH (µU/ml)</td>
<td>90 ± 2</td>
<td>100 ± 4</td>
</tr>
<tr>
<td>Pool</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µU/100 g body weight)</td>
<td>614 ± 43</td>
<td>645 ± 42</td>
</tr>
<tr>
<td>Disposal rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µU/100 g/min)</td>
<td>18.1 ± 1.9</td>
<td>18.4 ± 2.4</td>
</tr>
<tr>
<td>Plasma T4 (µg/dl)</td>
<td>5.3 ± 0.5</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Plasma T3 (ng/dl)</td>
<td>84 ± 8</td>
<td>85 ± 6</td>
</tr>
</tbody>
</table>

¹ Number of animals. Pool and disposal rate were studied in 4 EB-treated hypothyroid rats.
² P < 0.05, ³ P < 0.005, ⁴ P < 0.001 vs control euthyroid rats, respectively.
⁵ Compares EB-treated vs the respective control group. Only significant differences are shown.
Results

[125i]rTSH kinetics

Values for various aspects of the peripheral turnover of [125i]rTSH in control and EB-treated rats are shown in Table 1. In control euthyroid animals the fractional turnover rate averaged 3.0 ± 0.3 (mean ± SEM) %/min, and the distribution space 6.8 ± 0.5 ml/100 g body weight; as a consequence, the mean MCR was 0.20 ± 0.03 ml/100 g body weight. Mean value for plasma concentration of TSH was 90 ± 2 μU/ml, for its extrapituitary pool was 614 ± 43 μU/100 g body weight and for disposal rate 18.1 ± 1.9 μU/min/100 g body weight. After the administration of oestrogens these parameters remained essentially unchanged. When compared with control euthyroid rats, the control hypothyroid rats had a decrease in the fractional turnover rate (P < 0.05) and an increase in the distribution space of TSH (P < 0.005); consequently the MCR was unaltered. However, extrapituitary pool and disposal rate were both significantly increased (P < 0.001). In hypothyroid rats treated with EB there was an augmentation in distribution space (P < 0.025) as compared with control hypothyroid animals. Other parameters remained unaltered.

Pituitary-thyroid axis

Results are depicted in Fig. 1. Concordant with a previous work (Boado et al. 1983a) oestrogens reduced the pituitary TSH in euthyroid rats (P < 0.001) and increased pituitary weight (P < 0.01) and thyroid weight (P < 0.05). While in euthyroid rats oestrogens failed to alter plasma TSH, in hypothyroid animals oestrogens induced a sharp rise in plasma TSH from the already high concentrations found in untreated hypothyroid controls (P < 0.01). There was a significant decrease in pituitary TSH in EB-treated hypothyroid rats as compared with control hypothyroid animals (P < 0.005). The magnitude of the decrease was comparable to that produced by EB in euthyroid rats. In hypothyroid animals oestrogens also increased pituitary weight (11.0 ± 1.3 vs 20.5 ± 2.6 mg, P < 0.025) and pituitary proteins (0.67 ± 0.07 vs 1.26 ± 0.22 mg/gland, P < 0.05).

TRH test

Fig. 2 depicts the increments in plasma TSH 10 min after iv administration of TRH. Oestrogen-treated rats had a higher response to TRH than control animals (P < 0.001).

Effects of EB on plasma and pituitary TSH levels in euthyroid and hypothyroid female rats. Animals were treated with 25 μg EB/100 g body weight during 9 days. Each value represents the mean ± sem (vertical bar). The numbers in parantheses indicate the number of animals studied in each group. For details see Material and Methods.
Effects of EB on the TSH response to TRH in euthyroid rats. Animals were treated with 25 μg EB/100 g body weight during 9 days. Blood samples were drawn before and 10 min after the injection of 1 μg TRH/100 g body weight. Each bar represents the mean ± sem. Ten EB-treated and 10 control rats were studied.

Discussion

The present data suggest that the marked decrease of pituitary TSH in the presence of normal plasma TSH concentration induced by EB in euthyroid rats is unrelated to the peripheral kinetics of TSH. These findings would correlate with previous observations that changes in serum concentration of TSH in different pathological states are not related to alterations in the peripheral metabolism of this hormone, but are rather due to changes in hormone secretion. This can be exemplified by the augmentation of TSH release in diabetes insipidus (Fujimoto & Hedge 1982) or by the decrease in release observed in diabetes mellitus (Pastor & Jolín 1983). The present work shows that control hypothyroid animals had a significant decrease in the fractional turnover rate of TSH from euthyroid controls, which was counter-balanced by an expansion of the distribution space, resulting in a normal MCR. However, plasma concentration and extrapituitary pool of TSH were both significantly increased, leading to a marked augmentation in degradation rate of this hormone. The administration of oestrogens failed to induce changes in control parameters of euthyroid rats. In hypothyroid rats, oestrogen treatment was followed by a significant rise in distribution space and plasma concentration of TSH. Both these changes resulted in a non-significant increase in the peripheral pool.

As in the case of euthyroid rats, the final hormone degradation was normal.

Previous reports have shown an oestrogen-induced hypertrophy of the pituitary gland with an increase in pituitary weight (D'Angelo 1968) and in the pituitary content of proteins, ADN and ARN (Kapp & Fox 1981), whereas these parameters were not altered by testosterone (Kapp & Fox 1981). In the present work EB increased the pituitary content of proteins as well as the weight of the pituitary and thyroid glands, probably as a result of an anabolic effect (Underwood & Van Vyik 1981).

Hypothyroid rats are known to have an increased number of pituitary receptors sites for TRH and that these receptors increase further with the administration of oestrogens (De Lean et al. 1977). Such changes are associated with an augmented response of TSH to TRH (De Lean et al. 1977; Chen & Walfish 1978). Therefore, it seems reasonable to speculate that in the absence of suppressive effects of thyroid hormones in hypothyroid rats, the oestrogen-induced augmentation of pituitary TRH receptors may have contributed to accelerate the already faster than normal release of TSH. This increased hormone supply to the circulation, in association with a normal TSH disposal rate, might have led to the significant elevation of plasma TSH. Along the same line it can be speculated that with a normal TSH releasing mechanism in euthyroid animals, the administered oestrogens could have accelerated the release of TSH from a contracted pituitary TSH pool so as to maintain plasma TSH within physiological levels. An adjusting mechanism such as this seems reasonable from the knowledge that the total circulating pool of TSH in euthyroid rats barely represents 0.5% of the total pituitary pool of this hormone; consequently, even major changes in pituitary TSH content would not necessarily be reflected in significant alterations of the circulating hormone providing the normality of the TSH releasing mechanism. This contention seems supported by the fact that hypothyroid rats have a several-fold elevation of serum TSH in the presence of normal or even subnormal pituitary TSH pool (Silva & Larsen 1978; Boado & Zaninovich 1983).

More studies are needed to clarify the mechanism of action of EB on pituitary TSH. It seems from the present data, however, that the normal plasma TSH in the presence of low pituitary TSH induced by oestrogens in euthyroid rats (Boado et al. 1983) was not due to alterations in...
peripheral turnover of this hormone, and that the similar pituitary effect of oestrogens in hypothyroid rats was most likely the result of an acceleration in the release of TSH.

References


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