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Effects of estradiol benzoate on 5′-iodothyronine deiodinase activities in female rat anterior pituitary gland, liver and thyroid gland

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Abstract

There is little information on the possible effects of estrogen on the activity of 5'-deiodinase (5'-ID), an enzyme responsible for the generation of T3, the biologically active thyroid hormone. In the present study, anterior pituitary sonicates or hepatic and thyroid microsomes from ovariectomized (OVX) rats treated or not with estradiol benzoate (EB, 0.7 or 14 µg/100 g body weight, *sc*, for 10 days) were assayed for type I 5'-ID (5'-ID-I) and type II 5'-ID (5'-ID-II, only in pituitary) activities. The 5'-ID activity was evaluated by the release of ^{125}I from deiodinated ^{125}I rT3, using specific assay conditions for type I or type II. Serum TSH and free T3 and free T4 were measured by radioimmunoassay. OVX alone induced a reduction in pituitary 5'-ID-I (control = 723.7 ± 67.9 *vs* OVX = 413.9 ± 26.9; $P<0.05$), while the EB-treated OVX group showed activity similar to that of the normal group. Thyroid 5'-ID-I showed the same pattern of changes, but these changes were not statistically significant. Pituitary and hepatic 5'-ID-II did not show major alterations. The treatment with the higher EB dose (14 µg), contrary to the results obtained with the lower dose, had no effect on the reduced pituitary 5'-ID-I of OVX rats. However, it induced an important increment of 5'-ID-I in the thyroid gland (0.8 times higher than that of the normal group: control = 131.9 ± 23.7 *vs* OVX + EB 14 µg = 248.0 ± 31.2; $P<0.05$), which is associated with increased serum TSH (0.6-fold *vs* OVX, $P<0.05$) but normal serum free T3 and free T4. The data suggest that estrogen is a physiological stimulator of anterior pituitary 5'-ID-I and a potent stimulator of the thyroid enzyme when employed at high doses.

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- Thyrotropin

The effects of estrogen on the hypothalamic-pituitary-thyroid axis are not well understood. Data from some studies have suggested that sex steroid hormones may modulate the 5'-iodothyronine deiodinases (5'-ID) (1-3), enzymes responsible for converting thyroxine (T4) to triiodothyronine (T3), the biologically active hormone (4). Based on several functional criteria, 5'-deiodinases are classified into two isoenzymes: type I (5'-ID-I) and type II (5'-ID-II). Type I 5'-ID enzyme is most abundantly found in the

liver, kidney and thyroid and seems to be responsible for generating most of the serum T3 (4). In the case of 5'-ID-II, most activity is found in the central nervous system, pituitary, and brown adipose tissue, where it appears to catalyze the local T4 to T3 conversion (4). It has been reported that estradiol administered to ovariectomized (OVX) rats increased serum T3 and decreased serum T4 (1) in a dose-dependent manner. The hepatic T3 generation has been shown to be increased in ovariectomized rats treated with higher but not lower doses of estradiol (2). These data suggest that estrogen stimulates the peripheral T4 to T3 conversion by increasing the hepatic 5'-ID-I. However, other investigators (3) did not detect any *in vivo* or *in vitro* effect of estrogen on hepatic 5'-ID-I activity or on its mRNA. Therefore, the effect of estrogen on the hepatic 5'-ID-I remains controversial. To our knowledge, there are no reports on the effects of estradiol on 5'-deiodinase of other tissues. However, the possibility that sex hormones may regulate the pituitary gland enzyme has been suggested by Kohrle et al. (5) who showed a three times higher anterior pituitary 5'-ID-I activity and mRNA in female than in male rats.

The aim of the present study was to evaluate the effects of ovariectomy and the treatment of ovariectomized rats with different doses of estrogen on the 5'-ID activity in pituitary, thyroid and hepatic tissues.

Adult female Wistar rats weighing 200-250 g were maintained in a room under conditions of controlled light (12-h light and 12-h dark, lights on at 7:00 a.m.) and temperature (23/24ºC). All animals showed a regular 4-5 day estrous cycle monitored by vaginal cytology for at least two consecutive weeks before starting the experiments. Groups of rats were ovariectomized and a sham-operated group was used as control. The OVX rats were injected subcutaneously with 0.7 or 14 µg/100 g body weight estradiol benzoate (EB, Sigma Chemical Co., St. Louis, MO) daily for 10 days. One group of OVX rats and the control group received injections of vehicle instead of the hormone. All the rats in the control sham-operated group were at diestrus I on the day of sacrifice. The animals were sacrificed by exsanguination under ether anesthesia 24 h after the last injection and three weeks after ovariectomy. Blood was collected from the jugular vein and serum was obtained and stored at -20°C until assayed for TSH, free T3 and free T4. The pituitary, liver and thyroid were dissected out and processed for the determination of 5'-ID activity as described before (6,7). Briefly, the anterior pituitaries were homogenized in 50 mM Tris-HCl buffer, pH 6.8, sonicated for 15 s, and stored at -70ºC until assayed. The thyroids and livers were homogenized in the same buffer, centrifuged at 1,500 *g* (thyroid) or 15,000 *g* (liver) at 4ºC for 30 or 20 min, respectively. The supernatants of both tissues were ultracentrifuged at 190,000 *g* for 90 min and the pellets containing the microsomal fractions were resuspended and stored at -70ºC until assayed. Assays for 5'-ID were performed by a modification of methods described earlier (6,7). Pituitary sonicates (25 to 150 µg protein) were assayed for type I activity in 50 mM Tris-HCl, pH 6.8, in the presence of 2 nM rT3, 100 nM T4 and 400 mM DTT. For type II 5'-ID activity, pituitary sonicates (135-550 µg protein) were assayed in the same buffer in the presence of 2 nM rT3, 400 mM DTT and 10 mM PTU. Hepatic (35-270 µg protein) and thyroid (12-70 µg protein) microsome fractions were assayed for type I 5'-ID activity in phosphate buffer containing 1 mM EDTA, pH 6.9, in the presence of 1.5 µM rT3 and 100 mM DTT. Equal volumes of [^{125}I]-rT3 (Amersham, London, UK) purified before each set of assays by paper electrophoresis were added to each tube assay. Incubation in a shaking water bath at 37ºC was stopped after 1 h for pituitary, and 30 min for thyroid and liver by the addition of a mixture of 8% BSA and 10 mM PTU, fol-

lowed by cold 20% TCA. The samples were then centrifuged (2,000 rpm, 4ºC, 5 min), and 200 µl of the supernatant was applied to a Dowex 50 W-X2 column (100-200 mesh, hydrogen form; BioRad, Richmond, CA). ^{125}I eluted from the column with 10% acetic acid was measured with a gamma-counter. The specific enzyme activity is reported as femtomoles or picomoles of deiodinated rT3 h^{-1} mg $protein^{-1}$. Protein was measured by the Bradford method (8).

Serum TSH was measured by radioimmunoassay using NIDDK kits and is reported in terms of the reference preparation 3. Free T4 and free T3 were determined by radioimmunoassay using Coat a Count (DPC; Los Angeles, CA) kits. Standards were diluted 50% to facilitate measurement of the lower levels of the murine serum. All samples were measured in the same assay. Data are reported as means ± SEM. Data were analyzed statistically by one-way analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison test, with the level of significance set at $P<0.05$.

Ovariectomy decreased ($P<0.05$) the anterior pituitary 5'-ID-I activity and tended to decrease thyroid 5'-ID-I activity (Figure 1). Conversely, castrated rats chronically treated (10 days) with the lower dose (0.7 µg) of EB showed hepatic and thyroid 5'-ID-I activity similar to that observed for normal rats (Figure 1). Since we have previously demonstrated (9) that this same EB treatment brought the serum estradiol concentrations close to physiological levels, the normalization of the thyroid 5'-ID-I activity in the EB (0.7 µg)-treated castrated rats suggests that estrogen has a physiological stimulatory effect on 5'-ID-I in this tissue. Neither hepatic 5'-ID-I nor anterior pituitary 5'-ID-II showed significant changes with this EB treatment (Figure 1). At the same time, ovariectomy or 0.7 µg EB treatment did not significantly change serum concentrations of TSH, free T4 or free T3 (Table 1). However, there was a tendency to lower free T3 levels in the castrated group (Table 1), which would be consistent with a reduced thyroid 5'-ID-I, since in the rat this enzyme contributes significantly to serum T3 (10). Our results regarding serum thyroid hormones agree with some reports (11,12), and disagree with others (1,2) who demonstrated increased serum T3 and decreased serum T4 induced by estradiol treatment in ovariectomized rats.

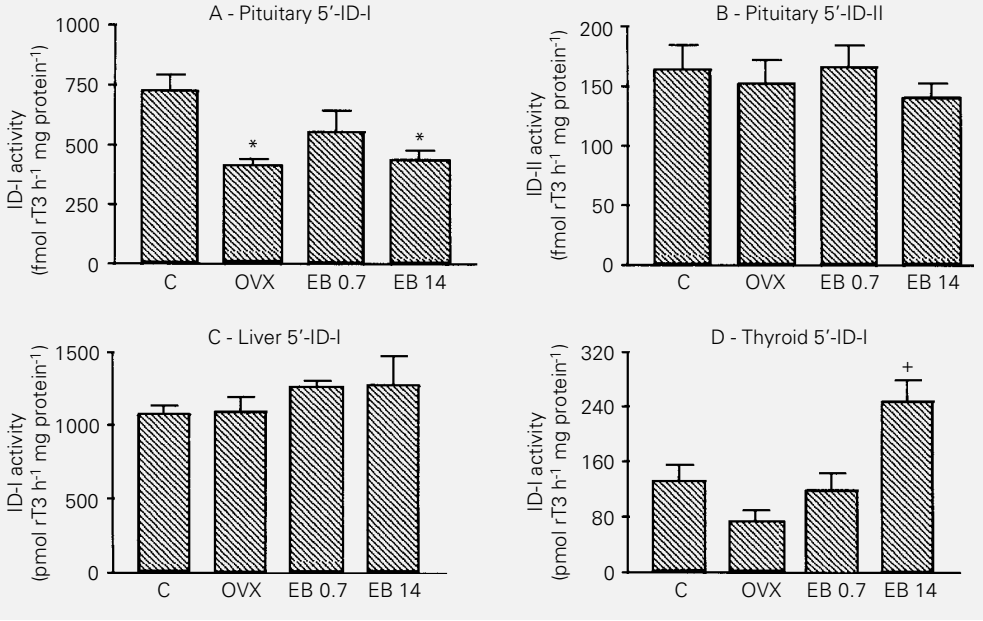

Figure 1 - Type I and II 5′-iodothyronine deiodinase activity in anterior pituitary homogenate (panels A and B), and type I 5′-iodothyronine deiodinase activity in liver and thyroid microsomes (panels C and D) of female sham-operated control (C) and ovariectomized rats treated with vehicle (OVX) or 0.7 or 14 µg/100 g body weight estradiol benzoate (EB), *sc*, daily for 10 days. Data are reported as means ± SEM. *$P<0.05$ *vs* C; +$P<0.05$ *vs* all groups (one-way ANOVA followed by Student-Newman-Keuls multiple comparison test).

Table 1 - TSH, free T3 and free T4 of ovariectomized rats (OVX) chronically treated with different doses (0.7 or 14 µg/100 g body weight) of estradiol benzoate (EB).

Data are reported as means ± SEM for the number of animals given in parentheses. *P<0.05 *vs* OVX (Kruskall-Wallis test followed by Dunn's multiple comparison test).

	Control	OVX	OVX + $EB_{0.7}$	OVX + EB_{14}
TSH (ng/ml)	1.94 ± 0.17 (7)	1.73 ± 0.10 (7)	1.96 ± 0.18 (7)	2.70 ± 0.28 (7)*
Free T4 (ng/dl)	0.64 ± 0.06 (7)	0.98 ± 0.22 (7)	0.59 ± 0.08 (6)	0.72 ± 0.06 (7)
Free T3 (pg/dl)	3.70 ± 0.60 (7)	2.28 ± 0.30 (7)	3.15 ± 0.40 (6)	3.08 ± 0.60 (7)

These controversial results may be related to the different treatment protocols and EB doses employed.

The results obtained by chronic treatment with the higher EB dose (14 µg) regarding 5'-ID activities showed another pattern. We have shown before (9) that with this treatment the rats become highly hyperestrogenized (17 times higher than controls at diestrus I). In the present study, we showed that the stimulatory effect observed with the lower EB dose on anterior pituitary 5'-ID-I was not seen with the higher EB dose (14 µg). In these hyperestrogenized castrated rats the anterior pituitary enzyme activity was reduced similarly to the ovariectomized group treated with vehicle (Figure 1). We have previously reported (9) a similar biphasic estrogen response regarding the *in vitro* TRH-stimulated TSH release and the TSH pituitary content of castrated rats, that was stimulatory at close to physiological doses and inhibitory at higher doses. Others (13,14) observed biphasic effects of estrogen on TSH secretion suggesting that estrogen increased serum TSH at lower doses and decreased it at higher doses. In our previous study (9), we did not find significant changes in the serum TSH of castrated hyperestrogenized rats, although there was a small tendency to higher values in this group. However, in the present study, the hyperestrogenized rats had significantly higher serum TSH (P<0.05, Table 1). Taken together, these data raise the hypothesis that the effects of estrogen are dose dependent and may have a temporal course. It is possible that in highly estrogenized castrated rats a higher rate of TSH secretion without an accompanying increase in the rate of TSH synthesis leads to reduction of TSH stores, that ultimately results in a decrease in TSH secretion. However, time course studies must be performed to substantiate this hypothesis. Pituitary type II deiodinase activity was not significantly changed by estrogen treatment, but tended to be lower in the hyperestrogenized group. This fact, taken together with the reduced activity of the type I enzyme, would be consistent with reduced intrapituitary T4 to T3 conversion and a consequent increase in TSH release.

In contrast to the effect on the pituitary gland, the treatment with the higher dose of EB induced an important increase in thyroid 5'-ID-I activity (P<0.05, Figure 1). This increase of the thyroid enzyme may be related to the high serum TSH observed in this group, since TSH is a well-known stimulator of 5'-ID-I in the thyroid gland (15). Recently, the presence of type II 5'-ID mRNA and activity has been reported in human thyroid glands (16). The enzyme activity and its mRNA were high mainly in the glands from Graves' patients and patients with follicular adenomas. Although rat FRTL-5 cells did not show the type II enzyme, it is possible that normal rat thyroids express 5'-ID-I. However, in the present study, the methodological approach used involved specific measurement of type I 5'-deiodinase, and did not allow us to make inferences about the

type II activity in the thyroid gland.

The present study does not elucidate the mechanisms responsible for these *in vivo* effects of high serum estradiol concentrations. A few reports have provided experimental evidence for a direct action of estrogen in the pituitary gland (14,17). However, the fact that serum TSH was increased while serum free T4 and free T3 remained normal may also suggest that these animals had a compensated hypothyroidism, i.e., estrogen would have a direct inhibitory effect on thyroid function that was compensated by the increased secretion of TSH. However, this hypothesis needs to be tested.

It has been shown that estrogen can increase renal iodine clearance (18). In our investigation, we cannot neglect the possibility that this fact may be responsible for the compensatory augmentation of serum TSH, which was verified in the hyperestrogenized ovariectomized rats. Nevertheless, early in human pregnancy, there is a small transitory decrease in serum TSH, even though the hyperestrogenism is associated with increased renal iodine clearance (18).

Liver 5'-ID-I did not show major alterations in the rats treated with estrogen, although there was a trend to a stimulatory influence of estrogen which has to be further investigated, since other authors (2) have suggested increased hepatic generation of T3 in castrated rats treated with estradiol, while others have shown no effect on the hepatic enzyme or its mRNA (3).

The increase in serum TSH caused by a high dose of estradiol cannot be attributed to changes in serum thyroid hormone-binding proteins, because transthyretin, which is the main thyroid hormone carrier protein in rat serum (19), is reduced in response to estrogen administration (20), which would lead to increased free thyroid hormones and consequently to reduced TSH secretion.

We have demonstrated for the first time dose-dependent effects of estrogen on the activity of type I 5'-deiodinases. At concentrations close to physiological levels estrogen stimulated the anterior pituitary and thyroid 5'-ID-I whereas high serum estrogen did not affect the reduced anterior pituitary activity of castrated rats, but induced a significant increase of the enzyme activity in the thyroid gland.

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