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ESTRADIOL BENZOATE INDUCED CHANGES IN PROTEIN SYNTHESIS AND LYSOSOMAL HYDROLASES IN THE PITUITARY OF MALE RATS

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In vitro protein synthesis, lysosomal hydrolases activity and peroxidase activity in the anterior pituitary were estimated in adult male rats treated with 50 µg of estradiol benzoate (EB) for 1 day or 7 days. Pituitary protein synthesis, protein and RNA content increased after 7 days. A significant increase in total and membrane-bound acid phosphatase was noted after 1 day or 7 days of EB treatment whereas total β-glucuronidase activity decreased in both 1 and 7 day group. Cathepsin activity increased after 7 days and pituitary peroxidase system did not change by EB treatment. These findings suggest that immediate change in the enzyme millieu may be one of the first reactions by which EB expresses its feedback control.

Exogenous administration of EB to normal adult male rats caused an increase in pituitary weight and decrease in pituitary LH secretion (1,2). In de novo synthesis studies, decreased incorporation of amino acid precursors into LH molecules was noted, however, synthesis of LH α subunit was stimulated by EB as indicated by accumulation of excess LH α subunit in the pituitary (3,4). It was apparent from these studies that EB was somehow affecting the pituitary subunit levels either by excess enzymic breakdown of newly synthesized LH molecules or by switching the whole machinery towards excess production of α subunits. In the present study we have investigated the effect of EB on pituitary total protein synthesis and on pituitary lysosomal hydrolases. It has been shown by several investigators that estrogen-induced growth of uterus

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is usually accompanied by induction of an enzyme hydrogen peroxidase oxidoreductase system (4,5). Since in pituitary also EB induces growth, signified by increased weight, it will be of interest to study whether such enzyme is existent in the pituitary and can be stimulated by EB.

METHODS AND MATERIALS

Adult male rats (180-200g b.w.) of Wistar strain were obtained from the animal facilities of our Institute. The animals were maintained on a lighting schedule of 14h light:10h darkness and food and water were available ad libitum.
Estradiol benzoate was injected subcutaneously daily at a dose of 50µg/rat/day in 0.1 ml oil. Control group received same volume of oil only. The animals were sacrificed after 1 day or 7 days of injection, the anterior pituitaries were separated out, weighed and used for different studies. The in vitro method used for estimation of protein biosynthesis was similar as used for gonadotrophin biosynthesis (6). In brief, quartered anterior pituitaries (4/flask) were incubated in leucine-free Eagle's minimum essential medium containing 20 µCi of (^{3}H)-leucine (New England Nuclear, sp. act. 50 mCi/mMol) in a Dubnoff's shaker at 37° C for 5hr. in an atmosphere of 95%O_2:5%CO_2 . The pituitaries were homogenised, centrifuged and dialysed to remove free (^{3}H)-leucine and counted with TritonX. Incorporation into newly synthesized protein was studied by precipitation of protein with 20% TCA, the precipitates were washed, dissolved in 0.1M NaOH and counted in liquid scintillation counter. For enzyme estimation, pituitaries of three animals were pooled, weighed and homogenised in 0.25M sucrose(3 pituitaries/ ml). The homogenates were centrifuged at 600g for 10mins, the supernatants were separated and recentrifuged at 105,000g in a Beckman Ultracentrifuge at 4° C for 45 mins. The 600g precipitates were resuspended and centrifuged at 20,000g and the pellets of this centrifugation were extracted with 1% TritonX in 0.25M sucrose, recentrifuged at 105,000g, and used as bound enzyme. Acid phosphatase (Orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) was assayed using M-sodium p-nitrophenyl phosphate as substrate and p-nitrophenol for standard curve (7). Beta glucuronidase (β-D-glucuronide glucurono hydrolase, EC 3.2.1.31) was estimated using phenolphthalein glucuronic acid Sodium salt (Sigma Chemicals, $2H_2O$/mole) as substrate(8). Cathepsin was assayed using 5% (W/V) gelatin and 0.07M cysteine as substrate (9). Peroxidase (Hydrogen peroxidase oxidoreductase, EC 1.11.1.7) activity was assayed using 1% O-dianisidine and 0.3%H_2O_2 as substrate, horse raddish peroxidase was used for standard (10).
Protein was estimated by the method of Lowry et al (11)using bovine-serum albumin as standard. RNA was estimated by the method of Schmidt and Thannhauser (12).
The data were analysed for statistical significance by Student's t test (13) .

RESULTS

Pituitary protein and RNA content following EB treatment :Pituitary protein and RNA content increased significantly after long-term (7 days) EB treatment (Table 1).

Table 1. Effect of 50 µg EB treatment on protein and RNA content of pituitary.

Treatment Group	Protein		RNA	
	mg/pituitary	µg/mg pituitary	µg/pituitary	µg/mg pituitary
Control	1.29±0.06	87.75±8.04	59.98± 4.58	5.87±0.65
1day EB	1.54±0.29	98.41±8.72	69.77±13.69	8.20±1.30
	**	**	**	**
7dayE EB	1.75±0.08	131.75±6.3	116.87± 8.69	9.43±0.80

+ Mean ± S.E. These data represent the mean of three experiments and 18 pituitaries were used. ** $p < 0.01$

Effect of EB on the uptake and incorporation of (^{3}H)-leucine into TCA-precipitable protein : Table 2 presents the data for in vitro synthesis of proteins in 7 day EB-treated and control animals. As the data shows the uptake of (^{3}H)-leucine precursor by the pituitary and incorporation into the protein increased after 7 days of EB treatment. However, the ratio of incorporation : uptake remained unchanged, in both the groups. In the treated group a large proportion of newly synthesized protein was found in the media.

Changes in different enzyme activity of pituitary following EB administration :

Acid hydrolase activity : Specific activity of total and membrane-bound acid phosphatase in the pituitary increased significantly

Table 2. Effect of 50 µg EB treatment for 7 days on the uptake of (^{3}H)-leucine by the pituitary and incorporation into the TCA-precipitable proteins.

Treatment Group	UPTAKE (mMleucine/mg tissue x 10^{-11})			Incorporation (mMleucine/mg tissue x 10^{-11})		
	Pituitary	Media	Total	Pituitary	Media	Total
Control	4.75	1.86	6.63	4.38	0.63	5.01 (74.3%)
7day EB	5.58	2.92	8.50	4.60	1.7	6.3 (74.1%)

Data presented here is the average of two flasks, from two experiments. Number in parenthesis presents the percentage of precursor incorporated in protein.

following one day EB treatment and remained elevated all through 7 days of treatment. The percentage of free enzyme activity however decreased significantly. In a separate aliquot of the same sample, total β glucuronidase activity decreased after 1day of EB treatment, showing a significant decrease in the membrane-bound enzyme, whereas the concentration of free extracellular enzyme remained unchanged and it is reflected in significant increase in percentage of free enzyme (Table 3). Another acid hydrolase Cathepsin concentration did not change after short term treatment but increased significantly after 7 days of EB administration (Table 4).

Peroxidase activity :Pituitary peroxidase enzyme system was not affected by EB treatment. All through the treatment period the peroxidase activity was maintained at the same level (Table 4).

DISCUSSION

Estrogen administration has been shown to induce changes in pituitary structures and functions. Increase in pituitary size and weight as a result of chronic estrogen treatment has been reported previously by us in two separate short and long term

Table 3. Effect of short and long term EB treatment on pituitary acid phosphatase and β-glucuronidase activity.

Treatment Group	Acid phosphatase (U/mg protein)				β-glucuronidase (U)			
	Free	Bound	Total	%Free	Free	Bound	Total	%Free
Control	0.52± 0.02	1.02± 0.06	1.54± 0.05	33.7± 1.52	6.86± 0.10	15.60± 0.64	22.46± 0.73	30.6± 0.64
1day EB	0.63± 0.09	1.44** ± 0.06	2.07** ± 0.14	29.7± 2.98	7.22± 0.34	6.88*** ± 0.90	14.11*** ± 1.24	51.6*** ± 2.01
7day EB	0.42± 0.05	1.82** ± 0.24	2.24* ± 0.24	19.0** ± 3.32	5.56± 0.38	6.32*** ± 0.19	11.88*** ± 0.24	46.7*** ± 2.43

Data presented here are the Mean±S.E. of three sets of experiments representing 6 groups of 3 pituitaries.

*P< 0.05, **P<0.01, ***P<0.005

Acid phosphatase U = Enzyme liberates 1 µmol of P-nitrophenol/hour at pH 4.8 and 37°C.
β-glucuronidaseU= Enzyme liberates 1 µg phenolphthalein/hr/mg protein.

Table 4. Effect of EB on pituitary cathepsin and peroxidase activity

Treatment Group	Cathepsin Activity (U/mg protein)	Peroxidase activity (U/mg protein)
Control	17.32 ± 0.58	12.23 ± 2.03
1day EB	21.14 ± 2.52	9.37 ± 1.19
7day EB	30.47 ± 1.90**	9.08 ± 1.48

** $p<0.01$
Cathepsin Unit = Amount of enzyme which in 7 ml test solution causes an increase of OD to 1.0 in 10 minutes in excess of blank reading.
Peroxidase Unit = is the activity causing an increase in absorbency of 0.001/minute.

studies (1,14). In the present study a significant increase in RNA content, de novo synthesis of protein and protein content of pituitary was observed after 7 days of EB treatment. In the treated group, a large amount of newly synthesized protein was located in the media. Since EB has been shown to stimulate the pituitary prolactin production and release (15), a portion of the newly synthesized protein in the media and pituitary may be accounted for by increased prolactin secretion. In a previous study we also have observed that approximately 30% of the de novo synthesized proteins eluted at the area where prolactin elutes in a Sephadex G-100 column(3). In addition, earlier studies have shown that estrogen increases the RNA content of pituitary acidophils (16). These findings suggest that EB stimulates pituitary prolactin and other protein synthesis which ultimately causes pituitary growth.

The most important finding of this study is the way EB affected two lysosomal hydrolases - differentially and rapidly. Acid phosphatase activity increased significantly whereas β-glucuronidase activity decreased within 1 day of EB treatment. EB has been shown to affect the pituitary LH secretion in similar rapid fashion (2), when circulating LH level dropped significantly within few hours of EB treatment. Since acid phosphatase activity was found to be

concentrated in the basophilic cells of the pituitary (17), a correlation between high acid phosphatase and low LH production may exist as an inverse relationship between β-glucuronidase activity and prolactin secretion has been cited (18). From the present data, it will be interesting to hypothesize that the differential effect of EB on two enzymes may be due to its two different ways of treatment of lysosomes from two different cell types, gonadotrophs and mammotrophs, and this may be the initial step towards feedback mechanism.

Surprisingly, the third lysosomal enzyme cathepsin activity did not change until 7 days of EB treatment. Furthermore, EB seems to change the structural latency (19) and the permeability of the lysosomal membranes (20), this may be reason for the alterations in the profile of free and bound enzymes.

In the uterus, estrogen induces an enzyme peroxidase which in turn regulates the metabolism of estrogen and is said to be a specific marker enzyme for tissues displaying growth dependency on estrogen (4,5). Since pituitary has estrogen receptor system (21) and estrogen induces pituitary growth, we have monitored the pituitary peroxidase system. Present data shows evidence of presence of an peroxidase enzyme system in the pituitary, however, the dose of EB used in this study did not affect this enzyme

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