STIMULATION BY ESTRADIOL OF THYROTROPHIC HORMONE PRODUCTION IN ADENOHYPOPHYSEAL CULTURES OBTAINED FROM INTACT AND OVARIECTOMIZED RATS

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Estrogens play an important role in the regulation of synthesis and secretion of pituitary hormones: luteinizing (LH) and follicle-stimulating hormones (FSH) and prolactin [6, 7]. The connection between sex steroids and the thyrotrophic function of the pituitary has received little study, although the fact that women suffer from diffuse toxic goiter several times more frequently than men [1] suggests the existence of such correlations. So far as the direct action of estrogens on the secretion of thyrotrophic hormone (thyroid-stimulating hormone, TSH) is concerned, data in the literature on this question are ambiguous. Whereas some workers suggest that they have a direct effect [5], others consider that estrogens simply increase the sensitivity of thyrotrophs to hypothalamic thyrotrophin releasing factor [9, 13]. The direction of these effects may also be linked with sex differences and the dose of steroids administered [11, 12].

It was shown previously [4] that estrogens in vivo induce lasting intracellular changes in prolactin production by the adenohypophysis, which may persist and continue to be observed when the cells are transferred to conditions of growth in vitro.

In the investigation described below primary culture of adenohypophyseal cells was used to detect possible estrogen-dependent changes in the thyrotrophic function of the pituitary in rats and also to study the direct effect of estradiol in vitro on TSH secretion.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature female Wistar rats (150-200 g). Ovariectomy was performed on animals anesthetized with pentobarbital through a midline laparotomy. Starting with the 8th day

TABLE 1. Basal 3-Hourly Secretion of TSH in Cultures of Adenohypophyseal Cells from Intact and Ovariectomized Rats and Ovariectomized Rats Receiving Estradiol

No.	Group of rats	Age of culture, days	TSH concentration in medium, ng/mg protein		
			n	$M \pm m$	P
1	Intact	4	6	13,6±3,12	
2		7	6	3,4±0,45	
3		4	5	12,4±2,48	1—3>0,05
4	Ovariectomized	7	6	3,6±0,50	2—4>0,05
5	Ovariectomized and receiving estradiol	4	6	28,0±4,80	1—5<0,05 3—5<0,05
4		7	6	4,7±0,60	2—6>0,05 4—6>0,05

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TABLE 2. Effect of Estradiol Administered in vivo (Experiment 1) and in vitro (Experiment 2) on 24-Hourly Secretion of TSH in Adenohypophyseal Cell Cultures

No.	Group of rats	Age of culture, days	TSH concentration in medium, ng/mg protein		
			n	$M \pm m$	P
	Experiment 1				
1	Intact	3	5	170±28	1—2>0,05
2	Ovariectomized	3	6	104±15	1—3<0,01
3	Ovariectomized and receiving estradiol	3	4	372±30	2—3<0,001
	Experiment 2				
4	Control	4	6	94±20	4—5<0,02
5	17β-estradiol	4	6	227±40	

after the operation the rats received daily subcutaneous injections of 100 μg of an oily solution of estradiol dipropionate for 5 days. The completeness of ovariectomy and the action of estradiol were judged by examination of vaginal smears.

Cultures of adenohypophyseal cells from intact and ovariectomized rats (12 days after the operation) and from ovariectomized rats treated with estradiol were isolated by the method described previously [2]. The cells were cultured at 37°C in glass test tubes 16 mm in diameter in medium No. 199 with the addition of 20% embryonic calf serum during the first 2 days and 10% serum on the subsequent days of growth in vitro. Experiments were carried out on 3- and 7-day cultures. The medium in the tubes was replaced by fresh. The concentration of TSH in the medium was determined after 3 and 24 h and in the cells 3 h after the change of medium. In one of the experiments 17β-estradiol was added directly to the incubation medium for 24 h. The concentration of TSH in the medium and cell homogenates was determined by a radioimmunologic method [3] and expressed per milligram cell protein. Protein was determined by Lowry's method. The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

The results of basal 3-hourly secretion of TSH by adenohypophyseal cells in cultures obtained from intact and ovariectomized rats and ovariectomized rats treated with estradiol, are given in Table 1. The first fact to be noted is that in all the experimental groups hormone production decreased during growth of the cells in culture: after 7 days it was only one-quarter of its value on the 4th day after the beginning of growth in vitro. Such changes are evidently attributable to a progressive decline in the number of thyrotrophs in culture as has been shown by an immunohistochemical method [8].

Ovariectomy did not affect the basal secretion of TSH in 4- and 7-day cultures. Administration of estradiol to ovariectomized rats for 5 days led to an increase in TSH production. The rate of release of the hormone under these circumstances was higher not only than in cultures from ovariectomized animals, but also in those from intact rats. Changes in the basal TSH secretion in cultures from ovariectomized rats, treated or not treated with estradiol, persisted for 4 days of growth in vitro; later, differences in hormone secretion in the experimental groups were no longer significant.

Results of two experiments to study secretion by ovariectomized rats, treated or not treated with estradiol, in the course of 24 h and also during direct contact between the sex steroid and thyrotrophs in culture are given in Table 2 from the comparative aspect. In the first case the same rule can be observed as was described above. Nevertheless, it must be pointed out that a definite tendency was observed for the basal secretion of TSH to decrease in cultures from ovariectomized rats compared with cultures from intact animals; however, the differences were not statistically significant.

Following administration of estradiol to rats, TSH secretion by a culture of adenohypophyseal cells obtained from these animals was considerably higher than in the other groups. In another experiment, in which 17β-estradiol was added directly to the culture medium for 24 h, the quantity of hormone released was increased by 2.4 times (Table 2), evidence that estradiol acts directly on the thyrotrophic function of the adenohypophysis.

TABLE 3. Intracellular TSH Concentration in Cultures of Adenohypophyseal Cells Obtained from Intact and Ovariectomized Rats and Ovariectomized Rats Receiving Estradiol

No.	Group of rats	Age of culture, days	TSH concentration in cells, ng/mg protein		
			n	$M \pm m$	P
1	Intact	4	6	540±128	1—2>0,05
2	Ovariectomized	4	5	496±101	
3	Ovariectomized and receiving estradiol	4	6	198±37	1—3<0,05 2—3<0,05

Investigation of the TSH concentration in the thyrotrophs showed that preliminary ovariectomy did not affect this value, whereas administration of estradiol in vivo significantly reduced the TSH concentration in the cultured cells (Table 3). The low TSH concentration in the thyrotrophs was evidently the result of stimulation of secretory processes in the cells under the influence of estradiol.

The absence of estrogens in the experimental animals had no significant effect on the intracellular concentration or secretion of TSH by the pituitary, whereas an increase in the estrogen concentration stimulated TSH release considerably. Rivier and Vale [14] suggest that estrogens can stimulate the production of hypothalamic thyrotrophin releasing factor (TRF), which is known to be the main stimulator of TSH synthesis and secretion. Other workers consider that the mechanism of action of estrogens includes an increase in the sensitivity of thyrotrophs to TRF, possibly as a result of an increase in the number of receptors in the cells [9, 10]. The results of the present investigation do not disprove either of these suggestions. At the same time they show that increased concentrations of estrogens can modify the function of the hypothalamus—pituitary—thyroid system also through a direct effect on adenohypophyseal cells secreting TSH.

Finally, in endocrinopathies associated with estrogenization of the patient, the possibility not only of changes in the gonadotrophic and lactotrophic functions of the pituitary, but also of increased thyrotrophic activity and, as a result of this, enhanced thyroid activity, must be taken into account.

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