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EFFECT OF ESTRADIOL ON PROLACTIN SECRETION BY ADENOHYPOPHYSEAL CELLS OF INTACT AND OVARIECTOMIZED RATS IN PRIMARY MONOLAYER CULTURES

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Estradiol directly stimulated prolactin secretion by adenohypophyseal cells of intact rats in monolayer culture. Complex relations between estradiol and certain other regulators of the function of the pituitary lactotrophs were found. Changes in the synthesis and secretion of prolactin in animals with disturbed function of their gonads were shown to continue for 3 or 4 days during culture of adenohypophyseal cells *in vitro*.

KEY WORDS: culture of the adenohypophysis; prolactin; estradiol.

The pituitary hormone prolactin plays an important role in the regulation not only of lactation, but also of the function of the gonads. An increase in prolactin secretion due to the development of a pituitary adenoma or other factors is accompanied by hypogonadism in both women and men [8]. Meanwhile, the serum prolactin level varies depending on the estrogen levels. Experiments on rats have shown that ovariectomy is followed by a decrease in the prolactin concentration in the pituitary gland, and replacement therapy with estradiol restores the secretory activity of the lactotrophs [4]. Meanwhile many problems concerning the mechanism of action of estrogens on prolactin secretion have not been sufficiently thoroughly discussed in the literature.

The object of this study was to investigate the direct effect of estrogens on lactotroph function in a primary culture of the adenohypophysis, and the nature of their interaction with other regulators of prolactin secretion. Another aim was to compare the secretory activity of the lactotrophs *in vitro* during culture of cells obtained from experimental animals after ovariectomy, with or without replacement therapy with estradiol.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature and immature female Wistar rats weighing 150-200 g. Overiectomy was performed through a midline laparotomy incision on the animals anesthetized with pentobarbital. Seven days after the operation the rats were given 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 verified by examination of vaginal smears.

Cultures of adenohypophyseal cells from control, ovariectomized (12 days after the operation), and estradiol-treated rats were set up by the method described previously [1]. The cells were grown in sloping tubes 16 mm in diameter in medium No. 199 with the addition of antibiotics, 20% fetal calf serum for the first 2 days and 10% during the subsequent days of growth, in an atmosphere of 5% CO_2 and 90% air. Each tube contained 1 ml of culture medium. The experiments were carried out on 3- and 4-day cultures. After a change of medium, 24 h

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TABLE 1. Effect of Estradiol, TRH, dbcAMP, and Dopamine on Secretion of Labeled Prolactin (in cpm/mg protein) in Cultures of Rat Adenohypophysis

Test No.	Group	Prolactin in medium		
		$M \pm m$	n	P
	Expt. 1. 4-day culture, incubation for 3 h			
1	Control	7 484±328	5	
2	Estradiol	9 183±425	6	1—2<0,01
3	Dopamine	5 164±521	5	1—3<0,05
4	Estradiol plus dopamine	8 020±1345	5	1—4>0,05
5	TRH	10 369±1143	5	1—5<0,05
6	TRH + dopamine	8 793±589	5	1—6<0,05
7	Estradiol*	7 475±551	5	1—7>0,05
8	TRH*	8 011±887	5	7—8>0,05
	Expt. 2. 4-day culture, incubation for 24 h			
9	Control	40 500±2916	4	
10	Estradiol	101 027±5664	6	9—10<0,001
	Expt. 3. 3-day culture, incubation for 3 h			
1	Control	1 262±76	5	
2	dbcAMP	2 934±183	5	1—2<0,001
3	dbcAMP plus dopamine	2 179±248	4	1—3<0,01 2—3<0,05

*Preincubation with estradiol for 24 h, washing; No. 7) basal secretion for 3 h; No. 8) TRH for 3 h.

before the beginning of the experiment [^{14}C]leucine (Czechoslovakia, specific radioactivity 40 μCi/mmole) was added to the tubes in a dose of 5 μCi/ml. At the end of incubation with the isotope the tubes were washed four times with Hanks's solution with an excess of unlabeled leucine (50 mg%), after which 1 ml of medium No. 199 not containing serum was added for 3 h together with the following preparations: TRH (1 μg/ml), dopamine (0.5 μg/ml), crystalline estradiol (0.5 μg/ml) or sodium dibutyryl-cyclic AMP (dbcAMP, 2.5 mM). Labeled prolactin in the medium and cell homogenate was detected by electrophoresis in polyacrylamide gel. Stained regions of the gel, corresponding to the prolactin band were cut out, homogenized in 0.3% sodium dodecyl sulfate solution, transferred to scintillation jars with Bray's fluid, and their radioactivity was measured on an Intertechnique counter. The protein content in the cells was estimated by Lowry's method. The concentration of labeled hormone in the culture medium was expressed per milligram cell protein.

EXPERIMENTAL RESULTS

Experiments on 4-day cultures of adenohypophyseal cells revealed some special features of the action of regulators of prolactin secretion. As early as 3 h after its addition to the medium estradiol significantly increased prolactin secretion compared with the control (Table 1). Prolonged (for 24 h) incubation of the cells with the steroid led to an increase in the quantity of hormones secreted by 2.5 times. The relatively rapid effect of estrogens on prolactin liberation in the present experiments must also be noted. In experiments on a transplantable culture of GH_3 pituitary cells an effect of this sort was not found until a few days after addition of estradiol [3]. Cells of the GH_3 line obtained from pituitary tumors evidently lost much of their ability to react to estrogens in the course of *in vitro* culture for several years.

Dopamine, which is known to be one of the main inhibitors of prolactin secretion [5, 7], if added to the incubation medium, reduced basal secretion and completely abolished the stimulating action of estradiol (Table 1). It is interesting to note, however, that the inhibitory effect of dopamine against other stimulators of prolactin secretion (TRH and dbcAMP) was only partial. These observations may indicate a different mechanism of action of central (TRH) and peripheral (estrogens) stimulators of prolactin secretion.

According to information in the literature, the combined administration of TRH and estradiol gives an additive effect of stimulation of prolactin liberation by adenohypophyseal cells [6]. However, after prolonged incubation of cells with estradiol, following by washing and

TABLE 2. Basal 3-h Secretion of Prolactin (in cpm/mg protein) in Cultures of Adenohypophyseal Cells Obtained from Intact, Ovariectomized, and Sexually Immature Rats

No.	Group	Medium			Cells		
		$M \pm m$	n	P	$M \pm m$	n	P
	Expt. 1. 3-day culture						
1	Control	1910±183	6		9 041±535	5	
2	Ovariectomy	1070±116	6	1—2<0,01 1—3>0,05	3 998±469	6	1—2<0,001 1—3<0,001
3	Ovariectomy + estradiol	2539±291	6	2—3<0,001	5 200±468	6	2—3>0,05
	Expt. 2. 4-day culture						
1	Control	6250±339	6		9 255±983	6	
2	Ovariectomy	3060±116	6	1—2<0,001 1—3<0,001	3 464±458	6	1—2<0,001 1—3<0,02
3	Ovariectomy + estradiol	3240±96	6	2—3>0,05	5 927±430	6	2—3<0,01
	Expt. 3. 4-day culture						
1	Control (sexually mature rats)	5463±704	5		10 575±911	6	
2	Sexually immature rats	2320±334	6	1—2<0,01	5 775±317	6	1—2<0,001

incubation of the culture for 3 h with TRH, no increase in secretion was observed under the influence of the hypothalamic peptide (Table 1). Prolonged preincubation of the cells with the sex steroid evidently reduces their sensitivity to TRH.

Even more profound changes in the lactotrophic function of the pituitary was observed in experiments on adenohypophyseal cultures from ovariectomized rats. It will be clear from Table 2 that after culture of adenohypophyseal cells of ovariectomized rats for 3 and even 4 days the functional activity of the lactotrophs, including synthesis and secretion of the hormone, still remained at a low level. This level was comparable with the activity of lactotrophs obtained from sexually immature rats (Table 2). Prolonged administration of estradiol *in vivo* to castrated animals leads to an increase not only in prolactin secretion in a 3-day culture, but also to an increase in the total quantity of labeled hormone (medium plus cells), i.e., to its increased synthesis. In a 4-day culture the after-effect of estradiol administered *in vivo* on prolactin secretion was reduced somewhat, although the content of the hormone in the cells was still significantly higher than in the culture obtained from ovariectomized rats.

The fact that changes in synthesis and secretion of prolactin arising under the influence of estrogens *in vivo* were maintained for a long time after transfer of the cells to growth *in vitro* can evidently be regarded from two different viewpoints. First, depending on the estrogenic background (absence or excess of estrogens in the blood for a long time) the initial number of prolactin-secreting cells in the pituitary may change toward the time of isolation of the culture. Differentiation of lactotrophs from stem cells may take place; their number may vary likewise as a result of transformation of trophic cells of one type into another [2]. Second, it can tentatively be suggested that estrogens give rise to stable intracellular changes in the rate of synthesis and secretion of prolactin which persist for a long time even after transfer of the cells to growth *in vitro*.

The after-effect of estradiol *in vitro* could not be reproduced. During exposure of the adenohypophyseal culture for 24 h with the steroid the increased lactotroph function quickly returned to its initial level if the estradiol was removed from the medium (Table 1). It is possible, however, that contact for 24 h between cells and estradiol is too short to enable stabilization of the intracellular changes responsible for accelerated protein synthesis or differentiation and transformation of the cells in culture to take place. Further experiments involving longer incubation of the adenohypophyseal culture with estradiol will evidently answer the question of whether the changes observed in prolactin synthesis and secretion are the result of the direct action of estrogens or whether other regulatory factors also participate in the formation of stable changes in functional activity of the lactotrophs.

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