Estrogen-Related Changes in Anterior Pituitary RNA Levels[1] (36166)

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Physiological alterations in the uterus and vagina result from fluctuations in estrogen secretion during the estrous cycle (1, 2). In previous work, the anterior pituitary gland was shown to contain an estrogen-binding system similar to one present in the uterus (3). Since estrogen stimulates uterine nucleic acid and protein synthesis (2, 4), a similar effect might be expected in other target tissues such as the pituitary gland. We have examined this possibility by studying changes in pituitary nucleic acid and protein levels during the estrous cycle and by testing the effect of replacement therapy with ovarian steroids following ovariectomy. A significant amount of hypophysial RNA was found to be estrogen dependent.

Materials and Methods. Mature female rats, 2–3 months old, were obtained from the Charles River Breeding Laboratories and maintained under controlled conditions with 14 hr of light (0700 hr–2100 hr EDT) and 10 hr of darkness per day. Vaginal smears were taken between 0900 hr and 1100 hr to determine the regularity of the estrous cycle. Those exhibiting at least three regular estrous cycles were sacrificed by decapitation between 0900 hr and 1400 hr on different days of the cycle. The posterior pituitary was separated from the adenohypophysis and discarded. The adenohypophysis was weighed fresh to the nearest 0.2 mg, frozen in an acetone–dry ice bath at —70°, and stored frozen until analyzed for nucleic acid and protein content. Ovaries were examined for the condition of follicles and corpora lutea, and the uterus was trimmed and weighed intact. The oviducts were extirpated, compressed between two glass slides, and examined with a light microscope for the presence of ova. The following criteria were utilized to determine the stage of the estrous cycle (Table I):

Diestrus 1. Naked ova devoid of cumulus cells were present in the oviducts. The vaginal smear contained mostly leucocytes. Uterine weight (346 ± 8.4 mg) was low.

Diestrus 2. Uterine weight (395 ± 12.4 mg) slightly increased. The vaginal smear was predominantly leucocytic. No tubal ova remained from the previous ovulation.

Proestrus. No tubal ova. The vaginal smear contained nucleated epithelial cells and some cornified cells. Uterine weight (737 ± 53.4 mg) maximal; uteri distended (ballooned) with luminal fluid indicative of estrogenic stimulation (1). Ovaries had large follicles.

Estrus. Uteri were enlarged but not ballooned (480 ± 32.3 mg). Ovaries contained fresh corpora lutea. Tubal ova were clumped with adherent cumulus cells establishing that ovulation occurred the previous night. The vaginal smear contained sheets of cornified cells.

Ovariectomized rats received one of the following treatments on days 11–15 after ovariectomy: 1) control, 0.2 cc corn oil vehicle per day; 2) progesterone, 1 mg/day; and 3) 17β-estradiol, 1 μg/day. Steroids were administered subcutaneously in 0.2 cc oil. At autopsy on day 16, the uterine weight was determined, and anterior pituitaries were

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TABLE I. Autopsy Results for Cycling and Ovariectomized Rats.[a]

	Cyclic females				Ovariectomized 16 days		
	Diestrus 1	Diestrus 2	Proestrus	Estrus	Control	Progesterone (1 mg/day)	Estradiol (1 μg/day)
No. animals	21	18	18	15	5	5	5
Body wt. (g)	245 ± 5.3	257 ± 4.8	246 ± 5.4	245 ± 4.9	297 ± 4.9[b]	286 ± 6.8[b]	281 ± 8.0[b]
Uterine wt. (mg)	346 ± 8.4	395 ± 12.4	737 ± 53.4[b,c]	480 ± 32.3[b]	133 ± 13.7[b]	170 ± 3.6[b]	397 ± 22.8[d]
Pituitary wt. (mg)	11.2 ± .34	11.6 ± .36	11.4 ± .31	11.8 ± .38	12.7 ± .34	13.6 ± .67	17.0 ± .71[b,d]
No. ova	9.1 ± .8	0	0	10.2 ± .9	—	—	—

[a] Each value is the mean ± one standard error of the mean; [b] $p < .05$ vs. diestrus 1; [c] $p < .05$ vs. all other stages; [d] $p < .05$ vs. vehicle control.

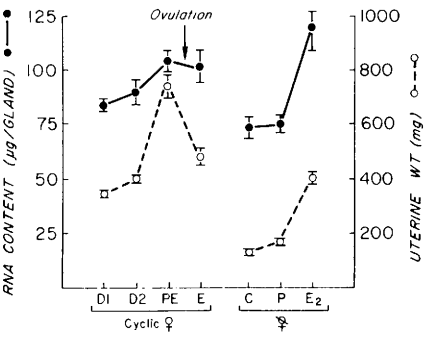

FIG. 1. Comparison of changes in pituitary RNA content with the relative estrogen level as indicated by uterine weight. D1 = diestrus 1, D2 = diestrus 2, PE = proestrus, E = estrus, C = oil vehicle, P = progesterone (1 mg/day), E_2 = 17β-estradiol (1 μg/day). Each point represents the mean ± the standard error of the mean.

recovered for measurement of protein and nucleic acid content.

The protein content of each pituitary was analyzed by the Lowry method (5). Nucleic acids were extracted and measured according to Scott *et al.* (6) using ultraviolet absorption at 260 mμ for the measurement of RNA and DNA. Purified bovine serum albumin (Sigma Chemical Co.), yeast RNA, and calf thymus DNA (Worthington Biochemical Corp.) were used as standards. Results were analyzed by the Student–Newman–Keuls test which permits a multiple comparison among means based on unequal sample sizes (7).

Results. Body and pituitary weights of cyclic females were not different (Table I). Uterine weight fluctuated during the estrous cycle and was greatest at proestrus (Fig. 1). After 16 days of ovariectomy, uterine weight was reduced and body weight was increased. Pituitary and uterine weights of ovariectomized rats were increased ($p < .05$) by 5 days of estradiol treatment, while progesterone had no significant effect on the parameters measured.

In cyclic females, there was a progressive increase in the content and concentration of pituitary RNA from diestrus 1 to proestrus (Fig. 1, Table II). Pituitary protein and DNA levels did not fluctuate significantly during the cycle, and the same was true for the RNA/DNA and protein/DNA ratios.

TABLE II. Anterior Pituitary Nucleic Acid and Protein Levels in Cyclic Female Rats and Ovariectomized Rats Following Replacement Therapy with Progesterone or 17β-Estradiol.[a]

	Cyclic Females				Ovariectomized 16 days		
	Diestrus 1	Diestrus 2	Proestrus	Estrus	Control	Progesterone (1 mg/day)	Estradiol (1 μg/day)
No. pituitaries	21	18	18	15	5	5	5
Content (μg/gland)							
Protein	479 ± 43.1	556 ± 76.6	639 ± 65.2	633 ± 80.7	654 ± 63.6	626 ± 53.6	892 ± 131.6[b]
RNA	84 ± 3.3	90 ± 5.6	104 ± 5.2[b]	101 ± 7.8	73 ± 5.0	75 ± 4.4	118 ± 9.8[b,c]
DNA	54 ± 4.0	54 ± 5.1	61 ± 3.8	59 ± 4.3	68 ± 3.5[b]	68 ± 5.7	81 ± 6.9[b]
Concentration (μg/mg)							
Protein	42.6 ± 3.7	48.3 ± 6.0	55.8 ± 5.3	52.5 ± 5.6	52.1 ± 5.9	46.1 ± 3.2	52.4 ± 7.1
RNA	7.5 ± .27	7.9 ± .52	9.1 ± .35[b]	8.5 ± .49	5.8 ± .38[b]	5.5 ± .27[b]	6.9 ± .39
DNA	4.7 ± .30	4.6 ± .44	5.4 ± .32	5.0 ± .27	5.4 ± .36	5.0 ± .24	4.8 ± .33
RNA/DNA ratio	1.55 ± .17	1.69 ± .28	1.71 ± .22	1.70 ± .09	1.06 ± .03[b]	1.12 ± .06[b]	1.44 ± .03[c]
Protein/DNA ratio	8.8 ± .63	10.6 ± 2.04	10.2 ± .70	10.3 ± .73	9.5 ± 4.6	9.3 ± .64	10.8 ± .80

[a] Each value is the mean ± one standard error of the mean; [b] $p < .05$ vs. diestrus 1; [c] $p < .05$ vs. vehicle control.

In ovariectomized rats, progesterone replacement therapy did not influence pituitary protein and nucleic acid levels (Table II), whereas estradiol treatment increased ($p < .05$) the glandular RNA content and the RNA/DNA ratio.

By comparing the pituitary nucleic acid and protein levels of cyclic and ovariectomized females (Table II), it can be seen that the DNA content increased and the concentration of RNA decreased following ovariectomy. This resulted in a significant reduction in the RNA/DNA ratio. Exogenous progesterone did not restore the pituitary RNA/DNA ratio of ovariectomized rats to the level observed during the cycle, but treatment with estradiol did.

Discussion. These results demonstrate a significant fluctuation in hypophysial RNA levels during the rat estrous cycle. Convey and Reece (8) also found a significant change in the pituitary RNA content during the rat estrous cycle. The present findings established that pituitary RNA increased prior to ovulation. This coincides with the time of active estrogen secretion (9) and the time of maximal uterine stimulation during the cycle (Fig. 1).

Using ovariectomized rats, it was shown that the cyclic changes in the pituitary RNA level could be attributed to stimulation by estrogen and not progesterone. Since the concentration of hypophysial RNA dropped following ovariectomy, a significant amount of the glandular RNA content appears to be estrogen dependent.

Previous studies have shown that estrogen increases the RNA content of pituitary acidophils (10, 11). Polysomes and ribosomes are numerous in prolactin cells following estrogen treatment (12, 13). Also, serum and pituitary prolactin levels are high at proestrus and estrus and decrease after ovariectomy (14–16). Treatment of ovariectomized rats with estrogen increases prolactin levels (15, 17). Since there is evidence that prolactin cells respond directly to estrogen (18), it is possible that the estrogen-related RNA changes observed in the anterior pituitary gland may be caused by a direct action of estrogen in the prolactin cell.

The demonstration of specific estradiol receptors in the pituitary cytosol fraction (19, 20), coupled with the evidence for estradiol concentration by the pituitary nuclear fraction (3, 21), indicates that the pituitary estrogen-binding system is similar to the uterine system. An early uterotrophic effect of estrogen is the stimulation of nuclear RNA synthesis (2, 4). The present results are consistent with the hypothesis that the pituitary estrogen-binding system is involved in the regulation of RNA synthesis.

Summary. Hypophysial RNA in the female rat was found to fluctuate during the estrous cycle. Pituitary RNA levels were highest at proestrus and lowest at diestrus 1. The concentration of pituitary RNA and the RNA/DNA ration decreased after ovariectomy. Estradiol (1 μg/day) restored the hypophysial RNA/DNA ratio of ovariectomized rats to a level comparable to that of cyclic females, whereas progesterone (1 mg/day) had no effect. It was concluded that the elevation in pituitary RNA at proestrus can be attributed to estrogen stimulation and that a certain portion of pituitary RNA is estrogen dependent.

1. Schwartz, N. B., Recent Progr. Horm. Res. **25**, 1 (1969).

2. Hamilton, T. H., Science **161**, 649 (1968).

3. Leavitt, W. W., Friend, J. P., and Robinson, J. A., Science **165**, 496 (1969).

4. Gorski, J., Toft, D., Shyamala, G., Smith, D., and Notides, A., Recent Progr. Horm. Res. **24**, 45 (1968).

5. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

6. Scott, J. F., Fraccastoro, A. P., and Taft, E. B., J. Histochem. Cytochem. **4**, 1 (1956).

7. Sokal, R. R., and Rohlf, F. J., "Biometry," p. 242. W. H. Freeman Co., San Francisco (1969).

8. Convey, E. M., and Reece, R. P., Proc. Soc. Exp. Biol. Med. **132**, 878 (1969).

9. Yoshinaga, K., Hawkins, R. A., and Stocker, J. F., Endocrinology **85**, 103 (1969).

10. Desclin, L., C. R. Soc. Biol. **133**, 457 (1940).

11. Wolfe, J. M., Amer. J. Anat. **85**, 309 (1949).

12. Pantic, V., and Genbacev, O., Z. Zellforsch. **95**, 280 (1969).

13. Zambrano, D., and Deis, R. P., J. Endocrinol. **47**, 101 (1970).

14. Sar, M., and Meites, J., Proc. Soc. Exp. Biol. Med. **125**, 1018 (1967).

15. Niswender, G. D., Chen, C. L., Midgley, A. R., Jr., Meites, J., and Ellis, S., Proc. Soc. Exp. Biol. Med. **130**, 793 (1969).

16. Amenormori, Y., Chen, C. L., and Meites, J., Endocrinology **86**, 506 (1970).

17. Chen, C. L., and Meites, J., Endocrinology **86**, 503 (1970).

18. Gersten, B. E., and Baker, B. S., Amer. J. Anat. **128**, 1 (1970).

19. Eisenfeld, A. J., Endocrinology **86**, 1313 (1970).

20. Kahwanago, I., Heinrichs, W. L., and Hermann, W. L., Endocrinology **86**, 1319 (1970).

21. Stumpf, W. E., Z. Zellforsch. **92**, 23 (1968).

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