Influence of the Estrous Cycle on the Nucleic Acid Content of the Rat Anterior Pituitary[1] (34327)

EDWARD M. CONVEY[2] AND RALPH P. REECE

Department of Animal Sciences, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey 08903

Substantial alterations in anterior pituitary weight and cytological staining reaction occur during the recurrent short estrous cycles of the rat. However, the cellular mechanisms by which these changes occur is unknown. Increased anterior pituitary weight may reflect an increase in cell numbers, an increase in cell size, or both. Pituitary deoxyribonucleic acid (DNA) concentrations and weight changes have been used to assess hypertrophy vs. hyperplasia in tumor bearing mice (1). Based on DNA measurements, Fand *et al.* (2) concluded that differences in weight of human pituitaries could be attributed to hyperplasia.

Cycle alterations in cytological staining reaction are correlated with the rhythmic secretion of anterior pituitary hormones (3, 4). Anterior pituitary rhythmicity is not intrinsic but is influenced by stimuli arising in the internal and external environment. These stimuli, in general, mediate their effects on the anterior pituitary via peptide neurohormonal substances which are produced within the neurosecretory cells of the hypothalamus (5). The primary role of these neurohumors is to promote the release of stored hormone, but there is evidence which suggests that neurohumors may also influence the rate of synthesis of new hormones (6, 7). Recent evidence suggests that some hormones may, as a primary event, activate ribonucleic acid (RNA) synthesis within the nucleus of their target cells (8). Whether hormone synthetic systems within the cells of the anterior pituitary are similarly controlled is not known.

In this investigation, anterior pituitary nucleic acids were measured to gain some insight as to the nature of the cellular changes occurring in the pituitary during the estrous cycle of the rat.

Materials and Methods. Adult nulliparous rats (170–220 g) of the hooded Norway strain were used in this study. They were maintained in colony cages under conditions of controlled temperature (70 ± 5°F) and lighting (12 hr of light daily). The diet consisted of Purina laboratory chow and water *ad libitum*. Vaginal smears were taken daily, at approximately 8:30 a.m., for at least three estrous cycles. Forty rats were selected on the basis of regular vaginal cycles and assigned at random (10 animals/group) to be killed on either the morning of proestrus, estrus, metestrus, or the first day of diestrus. All rats were killed by decapitation, their pituitaries were removed, and posterior pituitaries were discarded. Anterior pituitaries were weighed individually and stored frozen until analyzed for nucleic acids.

Thawed anterior pituitary glands were ground with a glass rod then cold extracted (ice bath) with 5 ml of chloroform:95% ethanol (3:1; v/v) for 2 hr followed by centrifugation (4°C) at 22,000*g* for 15 min. Precipitates were analyzed for DNA and RNA using the perchloric acid extraction procedure of Schmidt and Thannhauser (9) as has been routinely used in this laboratory for mammary gland tissue. RNA–ribose was quantified using the orcinol procedure of Mejbaum (10) as described by LePage (11). Optical density of the samples was read at 670 mμ. Pituitary RNA was calculated from

[1] Paper of the Journal Series, New Jersey Agr. Expt. Sta., Rutgers University—The State University of New Jersey, New Brunswick.

[2] Predoctoral Fellow of NIH. Present Address: Department of Dairy, Michigan State University, East Lansing.

TABLE I. Effect of Stage of Estrous Cycle on Anterior Pituitary (AP) Weight and Nucleic Acid Content.[a]

	Proestrus	Estrus	Metestrus	Diestrus
Body wt (g)	199.6 ± 4.69	197.6 ± 3.71	196.4 ± 4.63	208.5 ± 6.44
AP wt (mg)	8.82 ± 0.36[b]	10.57 ± 0.25	9.82 ± 0.32	9.32 ± 0.30[b]
DNA (μg/AP)	88.29 ± 2.42	92.07 ± 2.83	89.47 ± 2.99	90.66 ± 2.33
DNA (μg/mg of AP)	10.01 ± 0.33[c]	8.72 ± 0.22	9.12 ± 0.20	9.80 ± 0.33[c]
RNA (μg/AP)	65.43 ± 3.48[b]	76.83 ± 2.82	69.44 ± 2.84	59.93 ± 2.67[b]
RNA/DNA	0.74 ± 0.04[b]	0.84 ± 0.03	0.78 ± 0.02	0.69 ± 0.02[b]

[a] Values are the means ± standard error of means.

[b] Significantly less ($p < 0.05$) than the comparable estrous value.

[c] Significantly greater ($p < 0.05$) than the comparable estrous value.

a standard curve prepared with purified yeast RNA.[3] DNA was quantified using U. V. absorption at 268 mμ. DNA concentrations were calculated from a standard curve prepared with purified DNA.[4] Data were analyzed using analysis of variance, and comparisons of treatment means were accomplished using the method of least significant difference (12).

Results and Discussion. The results of this investigation reaffirm the recurrent alterations in weight of the anterior pituitary during the estrous cycle of the rat. The average weight of anterior pituitaries from rats killed during estrus (10.57 mg) was significantly greater ($p < 0.05$) than the comparable averages for rats killed during either proestrus (8.82 mg) or diestrus (9.32 mg) but not metestrus (9.82 mg). Changes in anterior pituitary weight were not reflected by alterations in total anterior pituitary DNA. Failure to detect concurrent changes in total anterior pituitary DNA is compatible only with the idea of cellular hypertrophy as the means by which these weight changes occur. This view is supported by the results of histological investigation which failed to detect mitotic figures in anterior pituitary cells of normal adult rats (13). Cellular hypertrophy is further indicated by significant alterations in mean cytoplasmic mass per cell (DNA concentration) during the estrous cycle. The average anterior pituitary DNA concentration (μg of DNA/mg of anterior pituitary tissue) at estrus (8.72) was significantly less ($p < 0.05$) than the corresponding averages for either proestrus (10.01) or diestrus (9.80) but not for metestrus (9.12).

In contrast to total DNA, changes in total RNA and RNA/DNA ratio of the anterior pituitary were correlated with alterations in gland weight during the estrous cycle. Anterior pituitaries from rats killed in estrus contained significantly more RNA and had a greater RNA/DNA ratio than did those from rats killed during either proestrus or diestrus. Although total RNA and RNA/DNA ratio decreased from estrus to metestrus, this decrease was not significant (Table I). The increase in anterior pituitary RNA and RNA/DNA ratio at estrus suggests a significant increase in protein synthetic activity. This increase may reflect a stimulative effect due to increased levels of circulating estrogens. Exogenous estrogen has been reported to increase the anterior pituitary content of RNA, but not of DNA, of female rats (14). Increased protein synthesis at estrus may reflect, at least in part, an increased demand for hormone production at this time. Wolfe (3) observed a degranulation of anterior pituitary cells beginning at proestrus and continuing throughout estrus in the rat. Such an outpouring of hormones would require increased protein synthesis to: (i) meet the demand for hormones by the peripheral systems, and (ii) replenish depleted anterior pituitary hormone stores.

Summary. Anterior pituitary glands from rats sacrificed during estrus were significantly

[3] Kindly supplied by Dr. F. F. Davis, Dept. of Biochemistry, Rutgers University, New Brunswick, New Jersey.

[4] Nutritional Biochemicals Corp., Cleveland, Ohio.

heavier than those from rats killed during either proestrus or diestrus but not metestrus. Failure to detect concurrent alterations in anterior pituitary DNA content indicated cellular hypertrophy as the means by which these weight changes occurred. Total anterior pituitary RNA and RNA/DNA ratio were significantly increased at estrus suggesting increased protein synthetic activity at this time.

1. Clifton, K. H., Cancer Res. **26**, 374 (1966).

2. Fand, S. B., Messineo, L., Ehmann, C. W., and Buscaglia, A. J., Proc. Soc. Exptl. Biol. Med. **125**, 192 (1967).

3. Wolfe, J. M., Anat. Record **61**, 321 (1935).

4. Catchpole, H. R., J. Endocrinol. **6**, 218 (1949).

5. Everett, J. W., Physiol. Rev. **44**, 373 (1964).

6. Talwalker, P. K., Ratner, A., and Meites, J., Am. J. Physiol. **205**, 213 (1963).

7. Sinha, D. K. and Meites, J., Endocrinology **78**, 1002 (1966).

8. Gorski, J., Noteboom, W. D., and Nicolette, J. A., J. Cell. Comp. Physiol., Suppl. **66**, 91 (1965).

9. Schmidt, G. and Thannhauser, S. J., J. Biol. Chem. **83**, 161 (1945).

10. Mejbaum, W., Z. Physiol. Chem. **258**, 117 (1939).

11. LePage, G. A., *in* "Manometric Techniques" (W. W. Umbreit, R. H. Burris, and J. F. Stauffer, eds.), Chap. 16. Burgess, Minneapolis, Minnesota (1957).

12. Steel, R. G. D. and Torrie, J. H., "Principles and Procedures of Statistics," McGraw-Hill, New York (1958).

13. Purves, H. D., *in* "Sex and Internal Secretions" (W. C. Young, ed.), Williams and Wilkins, Baltimore, Maryland (1961).

14. Kitay, J., Endocrinology **72**, 947 (1963).

Received July 11, 1969. P.S.E.B.M., 1969, Vol. 132.