

Radioimmunoassay Determinations of Gonadotropic Hormone Content in Different Regions of Male and Female Rat Adenohypophyses (38144)

S. J. SCHAFER¹ AND W. H. MCSHAN

Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706

It has been recognized that certain degrees of regionalization of cell types occur in the anterior pituitary gland. This regionalization varies among the species (1, 2). Histological ambiguity arose over FSH and LH cell type identification when Rennels (3) and Hildebrand *et al.* (4) proposed the FSH and LH cell type distributions were the reverse of those proposed by Purves and Griesbach (5).

Because the bioassay used by Kraicer and Logothetopoulos (2) did not distinguish between FSH and LH, it was of interest to assay pieces of rat anterior pituitary glands using radioimmunoassay (RIA). Then it could be determined whether the content of the gonadotropic hormones was localized in particular regions of the gland and whether such levels differed between the 2 hormones. The assumption made in formulating such a correlation between the content of hormone and regionalization of cell types is that intensity of specific histological or immunological stains for gonadotropic cell types is proportional to the amount of hormone contained within the cells.

Materials and Methods. Sixty-five day old diestrous female rats and 45 day old male rats (Holtzman Co., Madison, Wis.) were sacrificed in a carbon dioxide environment and the anterior pituitaries were dissected out and divided into pieces according to 1 of 3 categories: halves (caudal-cephalic), thirds (lateral A-medial-lateral B), or sixths (caudal and cephalic thirds).

Tissue pieces were weighed and homogen-

ized to known concentrations in 0.08M sodium barbital buffer (pH 8.6). After centrifugation at 4° and 8000 rpm for 30 min, the supernatant was assayed for LH and FSH activities. The RIA procedure was essentially as described by Beamer *et al.* (6), using the rat FSH and LH kits kindly provided by NIAMD. A 24 hr delay in addition of ¹³¹I-FSH (or LH) was used routinely. Mean values obtained per pituitary region after correction for weight differences were compared using Student-Newman-Keul's test (7).

Results and Discussion. Results, summarized in Tables I and II, indicate that the gonadotropic hormones are located throughout the glands in both male and female rats. This agrees with observations by Barnett *et al.* (8) and Swope *et al.* (9). The data, however, cannot be interpreted as to the problem of the one versus 2 cell type theory (8-11).

Although few significant differences existed among the various regions of the gland either for sex or hormone (Tables I and II), some trends of regionalization existed. Cephalic halves had more hormone than caudal halves and medial thirds had more than the lateral pieces (with one exception). Unlike the male glands where both medial pieces (No. 2, 5) had greater levels of FSH and LH, the cephalic medial piece (No. 2) of the female gland had higher levels of both hormones, but the caudal medial piece (No. 5) had lower levels of both hormones.

Areas of LH concentration paralleled areas of FSH concentration. The only exception was in the female where LH appeared to be somewhat more localized in the medial regions of the gland than was FSH.

¹Supported by Public Health Service Training Grant 5-T01-HD-00104-09 and the Ford Foundation Grant 630-0505A.

TABLE I. Gonadotropin Content in Regions of Female Rat Pituitary Glands as Determined by Radioimmunoassay.

| FSH | | | LH | | |
|---------------------------------------|-------------------------------------|----------------|------------------------|--------------------------------------|-----------------|
| Cephalic 9.2 ± 1.0 ^{a, b} | Lateral A 6.3 ± 0.5 | 1 3.8 ± 1.0 | Cephalic 63.7 ± 6.5 | Lateral A 36.9 ± 4.1 ^c | 1 28.0 ± 5.1 |
| Caudal 7.8 ± 1.6 | Medial 6.2 ± 0.2 | 2 5.2 ± 1.0 | Caudal 56.7 ± 7.3 | Medial 72.3 ± 4.8 ^c | 2 33.5 ± 5.7 |
| | Lateral B 9.6 ± 1.0 ^c | 3 3.1 ± 0.6 | | Lateral B 53.7 ± 2.3 ^c | 3 24.3 ± 2.4 |
| | | 4 3.0 ± 0.9 | | | 4 24.4 ± 5.4 |
| | | 5 2.2 ± 0.6 | | | 5 23.5 ± 2.8 |
| | | 6 3.9 ± 1.0 | | | 6 27.1 ± 1.5 |

^a μg NIAMD-FSH-RP-1/region ± SEM or μg NIAMD-LH-RP-1/region ± SEM.

^b Number of determinations: Halves (2 with 6 pituitaries pooled in each); thirds (2 with 7 pituitaries pooled in each); sixths (4 pituitaries assayed separately).

^c This value is significantly different (*P* < 0.05) from all other values within the group.

Glands were divided by estimating where cuts were to be made. This contributed to the overall variability. Moreover, such factors as

hormonal leakage from the cut edges of the tissue, losses during homogenization as well as assay and animal variability also con-

TABLE II. Gonadotropin Content in Regions of Male Rat Pituitary Glands as Determined by Radioimmunoassay.

| FSH | | | LH | | |
|--|--------------------------|------------------|---------------------------|--|-------------------|
| Cephalic 231.7 ± 0.7 ^{a, b, c} | Lateral A 168.7 ± 0.7 | 1 59.2 ± 21.3 | Cephalic 700.6 ± 139.5 | Lateral A 273.6 ± 16.0 ^c | 1 77.6 ± 17.0 |
| Caudal 174.3 ± 3.5 ^c | Medial 181.3 ± 0.7 | 2 93.4 ± 14.1 | Caudal 382.5 ± 87.9 | Medial 339.9 ± 14.1 | 2 158.4 ± 34.2 |
| | Lateral B 164.2 ± 7.4 | 3 64.7 ± 2.3 | | Lateral B 308.2 ± 6.4 | 3 126.2 ± 34.8 |
| | | 4 47.9 ± 10.7 | | | 4 88.5 ± 20.3 |
| | | 5 79.4 ± 0.36 | | | 5 144.8 ± 15.9 |
| | | 6 63.6 ± 4.33 | | | 6 113.1 ± 27.3 |

^a μg NIAMD-FSH-RP-1/region ± SEM or μg NIAMD-LH-RP-1/region ± SEM.

^b Number of determinations as in Table I.

^c Significance as in Table I.

tributed to overall variability. Thus, it could not be determined whether variability masked actual differences or whether the slight differences in concentration of hormone were not of biological significance.

The mean total content of the glands studied was in reasonable agreement with values reported in the literature (12-14) for rats of comparable ages.

Summary. Radioimmunoassay of regions of pituitary tissue suggested a tendency for the gonadotropic hormones to be more localized in the dorsomedial and cephalic regions of the gland. However, these hormones were present throughout the gland and whether the localization in particular regions was of biological significance could not be determined due to variability factors. The 2 hormones were generally located in the same regions of the gland, but evidence was not obtained to clarify whether both FSH and LH activities were present in one cell type.

1. Giroud, A., and Martinet, M., *Ann. Endocrinol.* **9**, 343 (1948).

2. Kraicer, J., and Logothetopoulos, J. *Endocrinology* **69**, 381 (1961).

3. Rennels, E. G., *Z. Zellforsch. Microsk. Anat.* **45**, 464 (1957).

4. Hildebrand, J. E., Rennels, E. G., and Finerty, J. C., *Z. Zellforsch. Microsk. Anat.* **46**, 400 (1957).

5. Purves, H. D., and Griesbach, W. E., *Endocrinology* **55**, 785 (1954).

6. Beamer, W. G., Murr, S. M., and Geschwind, I. I., *Endocrinology* **90**, 823 (1972).

7. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics," p. 110. McGraw-Hill Book Co., Inc., New York (1960).

8. Barnett, R. J., Ladman, A. J., McAllister, N. J., and Siperstein, E. R., *Endocrinology* **59**, 398 (1956).

9. Swope, A. E., Kahn, R. H., and Conklin, J. L., *J. Histochem. Cytochem.* **18**, 450 (1970).

10. Nakane, P. K., *J. Histochem. Cytochem.* **18**, 9 (1970).

11. Steinberger, A., Chowdhury, M., and Steinberger, E., *Endocrinology* **92**, 18 (1973).

12. Labhsetwar, A. P., *J. Reprod. Fert.* **20**, 21 (1969).

13. Labhsetwar, A. P., *J. Reprod. Fert.* **21**, 407 (1970).

14. Labhsetwar, A. P., *J. Reprod. Fert.* **22**, 165 (1970).