Sexual Differences in the Protein Content of the Hypothalamus in Rats[1]

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(Introduced by Prof. Virgilio G. Foglia)

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There is ample experimental evidence indicating that the different sexual pattern of gonadotropin secretion in the anterior pituitary is due to a direct influence of the hypothalamus (1). In a previous paper (2) it has been demonstrated that the cyclic control of gonadotropin secretion that characterizes female rats is accompanied by cyclic changes in the oxidative metabolism of the anterior and posterior hypothalamus, and that there are sexual differences in the oxygen uptake of this nervous structure. It has been proposed that the metabolic modifications of the hypothalamus in connection with sexual activity are related to changes in the synthesis and/or liberation of the gonadotropin-releasing factors (3). Considering the probable peptide nature of these hypothalamic substances which are implicated in the pituitary control of gonadotropin secretion (4), it was considered of interest to determine the DNA, RNA, and protein content of the different hypothalamic areas in male and female rats.

Material and Methods. Male and female albino rats fed on an adequate diet and weighing between 150 and 180 g were used. Light and temperature were controlled and kept constant (25°, 12 hr light and 12 hr darkness). Food and water were available *ad libitum*. Female rats were divided into groups according to the phase of the sexual cycle: (A) diestrus, (B) proestrus, (C) estrus. Vaginal smears were performed before sacrifice, and only rats with normal cycles were used.

The rats were sacrificed by decapitation, and the hypothalamus was removed. The sample was divided into three portions, anterior hypothalamus, middle hypothalamus, and posterior hypothalamus, as described previously (3). The samples were gently blotted on filter paper and weighed on a torsion balance. Separate 10 % homogenates were prepared with cold 0.32 *M* sucrose.

Nucleic acids were estimated by the method of Munro and Fleck (5) modified as follows: 0.5 ml of cold 10% TCA were added to 0.1 ml of tissue homogenate, allowed to stand for 10 min in the cold, and then centrifuged; the supernatant fluid was discarded, and the residue was washed once with 1 ml cold 10 % TCA and twice with 1 ml of ethanol–ether (3:1), the last wash being carried out at room temperature. The precipitate was resuspended in 0.3 ml of 0.3 *N* NaOH, incubated for 60 min at 37° and then chilled on ice. After the addition of 0.02 ml of cold 70 % perchloric acid, the tube was allowed to stand in the cold for 5 min and then centrifuged. The supernatant fluid was transferred to a 1-ml calibrated tube, and the residue was washed once with 0.6 ml of cold 1 *N* perchloric acid. This supernatant fluid was pooled with the first, the residue being saved to extract DNA. The volume of pooled supernatant fluid was made up to 1 ml with 1 *N* perchloric acid, and the absorbancy was measured at 260 mμ, using a model DB-G Beckman spectrophotometer; RNA was calculated using as a standard a solution of yeast RNA (Mann Research Laboratories) prepared un-

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der the same experimental conditions. DNA was extracted from the residue of the RNA extraction by adding 0.5 ml of 0.5 *N* perchloric acid, heating for 10 min at 70°, chilling on ice, and centrifuging. The supernatant fluid was transferred to a 1-ml calibrated tube, the residue was washed once with 0.2 ml 0.5 *N* perchloric acid, and the supernatant fluid was pooled with the first. The volume was adjusted to 1 ml with 0.5 *N* perchloric acid, and the absorbancy was determined at 264 mμ. DNA was calculated by using as a standard a solution of thymus DNA (Mann Research Laboratories) prepared under the same experimental conditions. Optimal conditions for the extraction of nucleic acid were checked by the orcinol and diphenylamine reactions.

Protein was determined according to Lowry *et al.* (6), using bovine albumin (Mann Research Laboratories) as the standard.

Results were analyzed for variance following Snedecor (7). The statistical significance of the data was determined according to Tukey's method (8).

Results. The protein content of different hypothalamic areas, in male and female rats, are summarized in Table I. It can be seen that the protein content of the anterior hypothalamus in male rats is higher than that of female rats, expressed either as milligrams per gram wet tissue or milligrams per milligram DNA. No significant differences were found in the protein content of the anterior hypothalamic area during the sexual cycle in the female rats, and the protein content of the middle and posterior hypothalamus was similar in both sexes.

Nucleic acids. Similar content of DNA in the three hypothalamic areas was found (Table II). There was no difference in RNA concentration, nor in the RNA/DNA ratio (Table III) of the anterior, middle, and posterior hypothalamus between the different groups.

Discussion. In a previous paper (9) it was demonstrated that the male and female types of hypothalamic control of pituitary gonadotropin function are associated with distinct metabolic patterns in the anterior hy-

TABLE I. Protein Content in Different Hypothalamic Areas.

	Anterior		Middle		Posterior	
	Protein (mg/g wet tissue)	Protein (mg/mg DNA)	Protein (mg/g wet tissue)	Protein (mg/mg DNA)	Protein (mg/g wet tissue)	Protein (mg/mg DNA)
A Male	92.4 ± 1.1[a] (11)	93.8 ± 3.54 (11)	88.4 ± 1.0 (11)	81.1 ± 3.07 (11)	83.2 ± 3.0 (10)	82.2 ± 3.72 (10)
B Diestrus	80.3 ± 1.8 (10)	76.6 ± 1.27 (10)	83.5 ± 1.0 (10)	84.2 ± 2.77 (10)	81.7 ± 1.0 (11)	80.4 ± 3.74 (11)
C Proestrus	80.5 ± 2.7 (12)	78.7 ± 2.08 (12)	86.0 ± 0.6 (11)	82.9 ± 2.21 (11)	83.2 ± 0.9 (12)	82.3 ± 2.71 (12)
D Estrus	83.0 ± 2.8 (11)	83.8 ± 3.08 (11)	84.2 ± 1.0 (11)	76.8 ± 3.06 (11)	83.1 ± 0.9 (11)	79.8 ± 2.66 (11)
Analysis of variance						
f ratio	6.61	7.42	2.28	1.37	0.46	1.17
p value	<.01	<.01	NS	NS	NS	NS
Multiple comparisons test						
$p < .05$ between	A vs B	A vs B				
	A vs C	A vs C				
	A vs D	A vs D				

[a] Mean ± standard error. Figures in parentheses are number of determinations. NS = not significant.

TABLE II. DNA Content in Different Hypothalamic Areas.[a]

	Anterior	Middle	Posterior
Male	0.99 ± 0.03[b] (9)	1.09 ± 0.07 (10)	1.06 ± 0.07 (9)
Diestrus	1.05 ± 0.09 (13)	0.99 ± 0.08 (13)	1.01 ± 0.07 (13)
Proestrus	1.02 ± 0.08 (10)	1.04 ± 0.35 (13)	1.01 ± 0.07 (8)
Estrus	0.99 ± 0.07 (13)	1.10 ± 0.08 (14)	1.04 ± 0.07 (13)
Analysis of variance			
f ratio	0.25	0.38	0.18
p value	NS[c]	NS	NS

[a] μg DNA/mg wet tissue.

[b] Mean ± standard error; number of determinations given in parentheses.

[c] NS = not significant.

pothalamus, and it was proposed that these metabolic findings in the hypothalamic area develop in response to sexual differentiation of the hypothalamus. Furthermore, there is considerable experimental evidence that the anterior hypothalamus is directly concerned with the sexual differences in the hypothalamic control of gonadotropin secretion (10).

The results of the present experiments show sexual differences in the protein content of the anterior hypothalamic area without modifications in the middle and posterior hypothalamus. Many functional and/or structural mechanisms could be responsible for this difference.

Considering the probable peptide nature of the hypothalamic-relasing factors (4), and taking into account that the cyclic and tonic control of gonadotropin secretion that characterizes female and male rats are produced by different mechanisms that exist in the anterior hypothalamus, it is probable that the sexual differences in metabolic activity of the anterior hypothalamus, described in a previous paper (2), and the protein content observed in the present paper, represent different regulating mechanisms that operate in the anterior hypothalamus of male and female rats.

RNA reflects the intensity of protein synthetic activity, and there is a well-established correlation between RNA content and the rate of protein synthetic activity (11). Since no changes were found in the RNA content in the anterior hypothalamus, it would appear that the sexual differences found in the protein content of this area are not due to difference in the rate of protein synthesis. However, unless ribosomal RNA is limiting, modifications in protein synthesis can occur without a measurable change in RNA content. Further evidence is needed before a conclusion can be reached in this respect.

No differences were found in the RNA and protein concentration in the different areas of hypothalamus during the sexual cycle in female rats. Considering the peptide nature of the releasing factors, these results appear to be in disagreement with several publications in which it has been demonstrated that the hypothalamic content of the gonadotropin-releasing factors in female rats undergoes cyclic modifications. No systematic discussion of this point is possible at present. Nevertheless, it can be postulated that the total protein content of hypothalamus could remain constant or with small modifications (not detectable by the methods used in this paper) even though the release and/or synthesis of the hypothalamic-releasing substances connected with gonadotropin secretion varies in each phase of the sexual cycle (12–14).

It is interesting to note that no changes were found in the DNA concentration per microgram of tissue of hypothalamus between the different groups. This fact indicates that the mean cytoplasmatic mass per hypothalamic cell is similar in all the experimental groups.

Summary. In the present paper the protein, RNA, and DNA content of anterior, middle, and posterior hypothalamus was studied in male and female rats. The protein content in the anterior hypothalamus is higher in male than in female rats. Similar values were observed in the middle and posterior hypothalamus between the groups. No sexual differences were found in the RNA and DNA content of the different hypothalamic areas. The relationships between these

TABLE III. RNA Content in Different Hypothalamic Areas.

	Anterior		Middle		Posterior	
	RNA (mg/g wet tissue)	RNA/DNA	RNA (mg/g wet tissue)	RNA/DNA	RNA (mg/g wet tissue)	RNA/DNA
Male	1.85 ± 0.09[a] (9)	1.91 ± 0.15 (9)	2.00 ± 0.07 (10)	1.88 ± 0.16 (10)	1.97 ± 0.06 (9)	1.96 ± 0.15 (9)
Diestrus	1.86 ± 0.06 (13)	1.82 ± 0.07 (10)	1.79 ± 0.06 (13)	1.88 ± 0.11 (13)	1.80 ± 0.06 (13)	1.87 ± 0.11 (13)
Proestrus	1.85 ± 0.08 (10)	1.87 ± 0.11 (10)	1.83 ± 0.06 (8)	1.79 ± 0.08 (13)	1.73 ± 0.13 (8)	1.72 ± 0.07 (8)
Estrus	1.83 ± 0.07 (13)	1.93 ± 0.11 (13)	1.89 ± 0.08 (14)	1.80 ± 0.09 (14)	1.85 ± 0.09 (13)	1.83 ± 0.09 (13)
Analysis of variance						
f ratio	0.05	0.36	1.32	0.22	1.08	0.56
p value	NS	NS	NS	NS	NS	NS

[a] Mean ± standard error. Figures in parentheses are number of determinations. NS = not significant.

findings and the modifications in the hypothalamic content of the gonadotropin-releasing factors is discussed.

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