Amino Acid Incorporation into Proteins of Anterior Pituitary Gland and Hypothalamus in Androgenized and Normal Female Rats[1] (35640)

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It is a well-established fact that gonadotropin secretion of the anterior pituitary gland (APG) is not identical in both sexes. Male rats secrete gonadotropins at a constant rate, while females have a cyclic secretion of these hormones. Strong evidence supports the concept that the sexual differences in gonadotropin secretion are due to a direct effect on the hypothalamus (1, 2).

The sexual differentiation of the hypothalamus into a cyclic or tonic pattern of gonadotropin secretion is influenced by gonadal hormones which act on the hypothalamus within a few days after birth. The administration of androgens during the first week of neonatal life to female rats induces a permanent masculinization of the hypothalamus which is evident in the subsequent regulation of pituitary secretion and of sexual behavior. Rats so treated show an anovulatory syndrome with a persistent estrus (1, 2).

It has been demonstrated that male and female types of hypothalamic control of pituitary gonadotropin function are associated with a distinct metabolic pattern in the hypothalamus; and the development of a specific metabolic entity in the anterior hypothalamus in response to sexual differentiation was postulated. In the prepuberal state, the hypothalamic metabolism of androgenized female rats is similar to that found in male rats (3).

To determine whether the modifications in the oxidative metabolism of hypothalamus, as well as in the pituitary secretion of gonadotropins of androgenized rats (4, 5) are accompanied by changes in the hypothalamic and pituitary protein synthesis, the *in vitro* incorporation of labeled phenylalanine into proteins of hypothalamus and APG was studied in androgenized rats. This study was extended to the variations in protein synthesis of these structures which accompany the estrus cycle in normal female rats.

Materials and Methods. Experiments were performed on female rats (weighing 150–170 g) from the strain of the Instituto de Fisiología. They were housed under conditions of constant temperature (23 ± 2°) and lighting (12 hr light and 12 hr darkness), fed *ad libitum* with a standard diet, and allowed free access to drinking water.

The animals were injected 3 to 4 days after birth either with testosterone propionate (androgenized group) or peanut oil (the other groups). Testosterone was injected subcutaneously at the dose of 1 mg dissolved in 0.1 ml of peanut oil. The following groups of rats were studied: (A) diestrus; (B) proestrus; (C) estrus; (D) metaestrus and (E) androgenized. Vaginal smears were performed during the morning before sacrifice. Only female rats with regular sexual cycles of 4–5 days as measured over a period of 2 months were used.

Animals were killed by decapitation and tissues were removed. Hypothalamus: the brain stem was placed on its dorsal surface and the entire hypothalamus was removed. The sample was limited rostrally by the anterior commissure and caudally by the end of the mamillary bodies; the superior border was limited by the thalamus-hypothalamic

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sulcus and the inferior border was taken as the line passing through the hypothalamic–pituitary junction. The following nucleus and areas were included in the studied sample: the anterior and preoptic hypothalamic areas, the paraventricular, suprachiasmatic, mamillary, posterior, arcuate, ventromedial and dorsomedial nucleus, as well as the median eminence. Pituitary: the posterior lobe was dissected away.

The samples were gently blotted on filter paper, weighed on a torsion balance and incubated in 1 ml of isotonic medium containing (μmoles): NaCl, 120; KCl, 5; K_2HPO_4, 1.3; $MgSO_4$, 1.3; $CaCl_2$, 1; Tris–HCl (pH 7.4), 33; glucose, 10; and 0.5 μCi of L-4 (^{3}H)-phenylalanine (20 Ci/μmole) obtained from the Commisariat a l'Energie Atomique (France). All chemicals were reagent grade. In the experiments in which time-course of incorporation was studied only 0.4 μCi/ml of labeled phenylalanine was used. Incubation was for 90 min at 37° with gentle shaking in a Dubnoff metabolic shaker. Gas phase was O_2:CO_2 (95:5, v/v). After incubation the tubes containing the tissues were rapidly removed and chilled in crushed ice, washed twice with saline solution, centrifuged in a refrigerated centrifuge, and homogenized (Potter-Elvehjem homogenizer) in 2 ml of 10% trichloroacetic acid (TCA) containing 0.2% unlabeled DL-phenylalanine (Sigma Co.). After the homogenization the suspension was centrifuged in a refrigerated centrifuge at 6500*g* for 15 min.

The TCA-insoluble residue was washed twice with 5% TCA, twice with chloroform–methanol (1:1), and once with 2 ml of ether; the precipitate was separated by centrifugation in the cold. The residue was then resuspended in 2 ml of 5% TCA and heated at 90° for 15 min; after cooling and centrifuging, the protein residue was dissolved in 0.5 ml of *N* NaOH, and aliquots were taken to determine protein by the method of Lowry *et al.* (6) using crystalline bovine serum albumin as a standard, and to measure radioactivity.

Radioactivity was determined with a Packard Tri-Carb liquid scintillation counter, and each sample was counted long enough to give a SE of less than 3%. Counts were corrected to 100% efficiency by the channels ratio method. The radioactivity incorporated into proteins was expressed as specific activity *i.e.*, dpm/mg of protein.

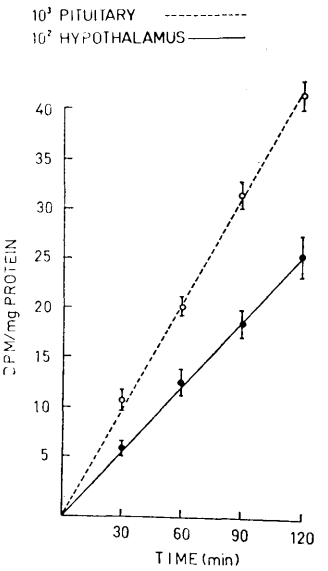

FIG. 1. Time-course of incorporation of L-(^{3}H)-phenylalanine into APG (4 separate determinations) and hypothalamus (5 separate determinations) proteins. The results are recorded as the mean ± SE and corrected for zero time radioactivity.

All results are presented as the means ± SEM of the number of experiments indicated in each case and analyzed for variance according to Snedecor (7). The significance of the data was determined according to Tukey's method (8).

Results. In preliminary experiments the time-course of incorporation was studied. The incorporation of labeled phenylalanine into proteins of hypothalamus and APG (Fig. 1) were linear during the 2 hr of incubation studied. Since similar curves were found in pilot experiments carried out in female rats during sexual cycle and in androgenized rats, 90 min of incubation was selected for further work.

Analysis of variance shows that there are significant differences in the specific activity of the hypothalamic and pituitary proteins formed in the different groups studied (Table I). Incorporation of labeled phenylalanine into proteins of the hypothalamus and the

TABLE I. Incorporation of L-(^{3}H)-Phenylalanine into Proteins of Anterior Pituitary Gland and Hypothalamus in Normal and Androgenized Female Rats.[a]

Groups	Protein radioactivity (dpm/mg of protein)	
	Anterior pituitary gland	Hypothalamus
A. Diestrus	20,015 ± 1500 (11)	4237 ± 413 (10)
B. Proestrus	31,573 ± 2108 (10)	7037 ± 600 (14)
C. Estrus	34,017 ± 2330 (9)	6900 ± 613 (9)
D. Metaestrus	20,371 ± 1018 (8)	3431 ± 277 (13)
E. Androgenized	22,147 ± 1660 (9)	4897 ± 863 (7)
Analysis of variance		
F ratio	8.76	11.5
p value	<0.01	<0.01
Multiple comparison test		
$p < 0.05$ between	A vs B	A vs B
	A vs C	A vs C
	B vs D	B vs D
	B vs E	B vs E
	C vs D	C vs D
	C vs E	C vs E

[a] Mean ± standard error. Values in parentheses are number of determinations.

APG were higher during proestrus and estrus than during diestrus and metaestrus. Protein synthesis in the hypothalamus and the pituitary of the group given androgen soon after birth was the same as in normal rats during diestrus and metaestrus, and always lower than in controls during proestrus and estrus.

Discussion. In previous papers (9–11) it has been demonstrated that the oxidative metabolism of APG and hypothalamus are modified in connection with changes in the secretion of sex hormones and gonadotropins and that the pituitary metabolic changes are directly related to the release of the sex hormones. On this basis, one can assume that the increased incorporation of phenylalanine into proteins of the APG is in some way connected with the changes in the oxidative metabolism of this gland. Whether the cyclic changes in oxygen uptake and protein synthesis of the pituitary gland are representative of modifications in the synthesis of hormones remain to be studied.

Cyclic changes in the oxidative metabolism of the hypothalamus have been demonstrated (9), higher values of oxygen uptake were observed during estrus and proestrus and lower ones during diestrus and metaestrus. A similar pattern of variations in the protein synthesis was observed in this study; the *in vitro* incorporation of labeled phenylalanine into hypothalamic proteins was higher in estrus and proestrus than in diestrus and metaestrus. It is probable that the changes in the oxidative activity of hypothalamus in female rats, as well as the cyclic modifications in protein synthesis described here, are in some way connected with the cyclic synthesis of the hypothalamic substances that control the pituitary gland in the female rats.

The androgenized rats showed similar levels of hypothalamic and pituitary protein synthesis when compared to those observed in diestrus animals, in spite of showing persistent estrus. Whether this pattern of amino acid incorporation into proteins is a consequence of the alterations in the gonadal secretion present in these rats or it is conditioned by the modifications in the sexual differentiation of hypothalamus resulting from the administration of testosterone soon after birth, must be elucidated. In this respect, it is interesting to note that these animals show metabolic alterations in the hypothalamus before the prepuberal state (3).

Summary. The *in vitro* incorporation of L-(^{3}H)-phenylalanine into proteins of the APG, and hypothalamus was measured in female rats given androgens soon after birth and compared with control animals during sexual cycle. Incorporation of labeled phenylalanine into proteins of the hypothalamus and APG were higher during proestrus and estrus than during diestrus and metaestrus. Protein synthesis in the hypothalamus and pituitary of androgenized rats was the same as in the normal group during diestrus and metaestrus, and always lower than in controls during proestrus and estrus.

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