Effect of Orchiectomy on the Amino Acid Incorporation into Proteins of Anterior Pituitary and Hypothalamus of Rats[1] (35441)

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(Introduced by Dr. Virgilio G. Foglia)
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The regulatory effects of gonadal steroids on the synthesis and release of gonadotropins, as well as the hypothalamic control of the pituitary functions have been established by several workers (1–3). Although there is no complete agreement about the effects of orchiectomy and gonadal steroids on the hypothalamic content of follicle-stimulating hormone releasing factor (FSH-RF) (1, 4, 5), evidence has been provided showing that orchiectomy and gonadal steroids affect the hypothalamic content of luteinizing hormone releasing factor (LH-RF) (6). In previous work it has also been shown that the oxidative metabolism of the anterior pituitary gland (APG) and the hypothalamus is markedly affected by orchiectomy (7–9).

Since it is generally accepted that a large number of homones control the metabolism of the target cells by regulating RNA and protein synthesis (10, 11), and taking into account the protein nature of pituitary gonadotropins, it seemed of interest to study the effects of orchiectomy and the testosterone supplement upon the *in vivo* protein synthesis in the APG, the hypothalamus and the cerebral cortex. This appeared particularly interesting, considering that at the present time there is no available information about the effects of these experimental conditions on the protein synthesis in the hypothalamus and the cerebral cortex, and that contradictory results have been reported in connection with the effect of orchiectomy on APG protein synthesis (12, 13).

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

Orchiectomy was carried out under ether anesthesia. Experiments were performed in two series: in the first the following groups of rats were studied simultaneously, (a) intact control, (b) 3 weeks orchiectomized rats, and (c) 3 weeks castrated rats treated with testosterone. The hormone was injected subcutaneously at the dose of 150 μg in 0.5 ml of sesame oil, twice a week, starting 1 day after operation and continuing throughout the experimental period. The second series of experiments had similar groups of animals as the first one, with the difference that 6-week castrated rats were used. Control and orchiectomized rats received only 0.5 ml of sesame oil.

L-4(^{3}H)-Phenylalanine (20 Ci/mmole) was obtained from the Commissariat a l' Energie Atomique (France), and all chemicals were reagent grade. Unless otherwise stated, at the scheduled date each animal received a subcutaneous injection of 0.2 ml of saline solution containing 8 μCi of labeled phenylalanine/100 g of body weight, being killed by decapitation 90 min later. Slices of the cerebral cortex, the whole hypothalamus and the APG (the posterior lobe was dissected away) were rapidly obtained in the cold and

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weighed. Cortical slices and hypothalamus obtained from each rat were individually processed, whereas 3–4 APGs were pooled in each experiment.

Tissues were treated in the cold for free amino acid extraction by homogenizing, in 2 ml, 10% trichloroacetic acid (TCA) containing 0.2% unlabeled DL-phenylalanine (Sigma Co.), allowed to stand in the cold for 15 min and centrifuged in a refrigerated centrifuge at 6.500*g* for 15 min. The TCA-insoluble residue was washed with 2 ml of the same TCA solution, and after pooling the supernatants the TCA was removed by washing with ether. The aqueous phase was evaporated to dryness under vacuum, and the residue was dissolved in 12 ml of Bray's solution for counting.

The TCA-insoluble residue was washed twice with 2 ml of chloroform–methanol (1:1) and once with 2 ml of acetone at room temperature, the precipitate was separated by centrifugation in the cold. The residue was then resuspended in 2 ml of 10% TCA and heated at 90° for 5 min; after cooling and centrifuging, the protein residue was dissolved in 0.5 ml of 88% formic acid, and aliquots were taken for protein determination by the method of Lowry *et al.* (14), using crystalline bovine serum albumin as a standard, and for measurement of radioactivity in vials containing 12 ml of Bray's solution.

Radioactivity was determined in a Nuclear Chicago 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 protein was expressed as sp act (SA), *i.e.*, dpm/mg of protein. The radioactivity in TCA-soluble fraction was expressed as dpm/mg wet weight of tissue, and served as an indication of the uptake of the labeled amino acid by the tissue.

All results are presented as the means ± SEM of the number of experiments indicated in each case. The results from intact controls were compared statistically with those of experimental groups by means of Student's *t* test, and differences were considered significant when *p* values were less than 0.05.

Results. In preliminary experiments the time course of incorporation was studied. For all the studied tissues the maximal incorporation of radioactivity into proteins occurred at 90 min after the injection; since similar kinetics was found in normal control and experimental groups, 90 min after injection of the labeled amino acid was selected for further work.

At both postoperation intervals, castration and substitutive therapy did not affect the uptake of labeled amino acid by the APG, hypothalamus, and cerebral cortex, since the values of TCA-soluble fraction in these tissues were essentially the same for all the studied groups.

The labeling of APG proteins was markedly affected by castration at both intervals, the SA of APG proteins from orchiecotmized rats was 30–31% higher than that from intact controls. In both cases, this increase was restored to normal values by testosterone treatment (Table I).

After 3 weeks of castration the changes in the hypothalamus differed markedly from those observed in the APG, and there was a significant drop (24%) in the SA of hypothalamic proteins. On the contrary, the lack of gonadal steroids for 6 weeks led to a significant increase of the hypothalamic protein synthesis, the SA of orchiectomized rats being 33% higher than that of intact controls. In both cases, the altered amino acid incorporation into hypothalamic proteins was restored to normal values by testosterone treatment (Table I).

Neither castration nor testosterone administration to castrated rats influenced the incorporation into cortical proteins (Table I).

Discussion. Considering that the *in vivo* administration of a labeled amino acid permits a comparison of the protein-synthetic activity in different tissues of the same animal, the results reported here clearly show that the APG has a higher protein synthetic activity than the hypothalamus and the cerebral cortex. This may be the reflection of the rapid synthesis and release of protein hormones.

It is interesting to point out that the protein-synthetic ability of two tissues closely involved in neuroendocrine functions like the

TABLE I. Effect of Orchiectomy and Testosterone Supplement on the Incorporation of L-(^{3}H)-Phenylalanine into Proteins of Anterior Pituitary Gland, Hypothalamus, and Cerebral Cortex.[a]

Time post-orchiectomy (weeks)	Groups	Protein radioactivity					
		Anterior pituitary gland		Hypothalamus		Cerebral cortex	
		(dpm/mg protein)	p value	(dpm/mg protein)	p value	(dpm/mg protein)	p value
3	Intact control	1839 ± 128 (5)		891 ± 62 (15)		904 ± 57 (11)	
	Orchiectomized	2398 ± 178 (5)	<0.025	680 ± 77 (17)	<0.05	955 ± 68 (10)	NS
	Orchiectomized + testosterone	1871 ± 229 (4)	NS	784 ± 80 (12)	NS	926 ± 60 (10)	NS
6	Intact control	1943 ± 172 (4)		879 ± 77 (11)		1017 ± 89 (11)	
	Orchiectomized	2544 ± 110 (4)	<0.025	1169 ± 133 (12)	<0.005	1069 ± 81 (11)	NS
	Orchiectomized + testosterone	2134 ± 168 (4)	NS	738 ± 54 (12)	NS	994 ± 99 (12)	NS

[a] The results are the means ± SEM of the number of experiments indicated in parentheses. Values from experimental groups were compared with those from intact control by means of the Student's t test, and p values are indicated when differences were significant; NS = nonsignificant.

hypothalamus and the APG is strongly affected by orchiectomy, in marked contrast with the lack of effect on the protein synthesis in the cerebral cortex. The fact that altered protein synthesis occurs without concomitant changes in the amino acid uptake by the tissues, would suggest that amino acid transport and protein synthesis are relatively independent processes, the hormone acting predominantly upon the latter.

Our results showing that orchiectomy leads to a significant increase of the APG protein synthesis agree with those from *in vitro* experiments reported by Tonoue and Yamamoto (12). However, Lee *et al.* (13) have found that castration has no effect on the *in vitro* amino acid incorporation into APG protein; we cannot explain at present the discrepancy between our results and those reported by the latter authors. The increase of APG protein synthesis which follows orchiectomy and the suppression of this increase by the administration of testosterone, clearly indicate that some of the metabolic features of the APG change as a result of the removal of the negative feedback by gonadal steroids.

It has been demonstrated that gonadotropin content of the APG increases following orchiectomy (1, 2, 15). Although the enhanced APG protein synthesis which follows orchiectomy might be considered as reflecting promoted biosynthesis of gonadotropins, it must be taken into account that amino acid incorporation into cell proteins is related to the biosynthesis of protein in general, but does not represent specifically the gonadotropin synthesis. Wakabayashi and Tamaoki (16, 17) have shown by immunochemical means that 1 week after castration only the biosynthesis of LH is increased, while after 2 weeks an increase in overall protein synthesis also occurs. Since the shorter postcastration interval studied in our work was 3 weeks, it is therefore probable that the increase in overall protein synthesis observed by us, besides indicating in part an increased synthesis of gonadotropins, is mainly the consequence of a cellular adjustment to a new physiological condition which is accompanied by hypertrophy (15) and increased oxidative metabolism (18).

Orchiectomy initially decreases and subsequently stimulates the hypothalamic protein synthesis, and both alterations can be cor-

rected by testosterone treatment. Although at present it is difficult to explain the actual mechanisms involved in the significant changes of hypothalamic protein synthesis produced by orchiectomy, the reported results deserve a further, although speculative comment. Evidence is now accumulating supporting the view that the hypothalamus rather than the pituitary is the sensitive site for the negative feedback by gonadal steroids, whereas there has also been demonstrated the existence of a negative short feedback mechanism exerted by FSH and LH and operating on the hypothalamus (1, 2).

Taking into account the progressive increase in the gonadotropin levels produced by orchiectomy, it is tempting to speculate about the possibility that the increased gonadotropin levels could be responsible for the changes in hypothalamic synthesis taking place 3 weeks after castration. In this connection, it is suggestive that in our laboratory it has been shown that 3–4 weeks after castration the cytochrome oxidase (EC 1.9.3.1) activity in the hypothalamus is markedly decreased (7), and that the administration of FSH or LH to hypophysectomized–castrated rats produces a strong depression in the oxidative capacity of the hypothalamus (18). Further experimental evidence is needed to clarify the relationships between the chanegs in the metabolic activity of the hypothalamus and its content of releasing factors.

Since 6 weeks after castration the pituitary and plasmatic levels of gonadotropins remain high (15), the increased hypothalamic protein synthesis is a finding that cannot be explained at present. One might suppose that the hypothalamic sensitivity to hormones changes at different intervals after castration, or that differences in the endocrine balance between 3 and 6 weeks after castration could affect in a different way the hypothalamic protein synthesis. It is therefore clear that more experimental evidence is needed before a meaningful conclusion can be reached.

Summary. The influence of orchiectomy upon the *in vivo* incorporation of labeled phenylalanine into proteins of rat anterior pituitary gland, hypothalamus, and cerebral cortex has been studied. The uptake of the labeled amino acid by all the studied tissues was unaffected by orchiectomy. Three and 6 weeks after castration there was a significant increase in the sp act of hypophyseal proteins, which was restored to normal values by testosterone administration. A biphasic response to orchiectomy was observed in the hypothalamus. After 3 weeks of castration, there was a significant decrease in the sp act of proteins, while an increase occurred after 6 weeks. In both cases testosterone administration corrected these altered patterns to normal values. Castration did not affect the protein synthesis in the cerebral cortex. The changes in the protein-synthetic activity are discussed on the basis of the neuroendocrine changes produced by castration.

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