CHANGES IN THE PROTEIN CONTENT IN HYPOTHALAMIC NEURONS OF NEONATALLY CASTRATED SEXUALLY MATURE MALE RATS

N. A. Borisova and K. Chandraseckhar

UDC 612.826.4.015.348-06:612.616.1

Changes in the protein content in neurons of the anterior and mediobasal hypothalamus of neonatally castrated sexually mature rats were demonstrated by an interferometric method. A considerable increase in the dry weight of the neurons was found in the medial preoptic region and the arcuate and ventromedial nuclei of the hypothalamus. The clearest changes were observed in the nuclei of these neurons. The results point to an influence of androgens in the period of sexual differentiation of the brain on hypothalamic structures responsible for the regulation of both the cyclic and the tonic secretion of gonadotropic hormones in sexually mature animals.

KEY WORDS: *hypothalamus; sexual differentiation of the brain; neonatal castration.*

The rat hypothalamus at birth is not yet sexually differentiated: In both females and males the structure of the anterior hypothalamus is potentially capable of developing into centers regulating the cyclic secretion of gonadotropin in adult animals [7]. The character of sexual differentiation of the hypothalamus is determined by the inducing effect of splenic hormones during a certain period of development. In rats this period begins at the end of embryonic development and extends over the first week of postnatal life [5]. Neonatal castration of males leads to differentiation of the hypothalamus according to the neutral type, the same as in females [11].

The mechanisms of sexual differentiation of the brain have not been explained. Dorner and Staudt [3] showed that the concentration of male sex hormones in the animal correlates with the size of the nuclei of neurons in the anterior and mediobasal hypothalamus. Differences in the size of the nuclei of neurons in the preoptic region and in the ventromedial hypothalamic nucleus are found very early: At birth in females the volume of the nuclei of the neurons is much greater than in males. These differences persist throughout the animal's life. If males were castrated on the first day after birth, the volume of the nuclei of the neurons in the preoptic region and in the ventromedial hypothalamic nucleus in the sexually mature state was close to that in females [3].

The writers showed previously [1] that the volume of the nuclei of neurons in the medial part of the preoptic region and in the suprachiasmal, arcuate, and ventromedial nuclei of the hypothalamus, i.e., the regions responsible for regulation of pituitary gonadotropic function, in sexually mature males castrated during the first week after birth was considerably greater than in intact males of the same age, and close to that in females.

The object of the present investigation was to study changes in the dry weight of neurons of the anterior and mediobasal hypothalamus in rats castrated during the period of sexual differentiation of the brain. Since the dry weight of a cell consists mainly of protein material, changes in the dry weight of the neurons could point to corresponding changes in protein metabolism.

Laboratory of Hormoral Regulation, Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 11, pp. 586-589, November, 1978. Original article submitted February 10, 1978.

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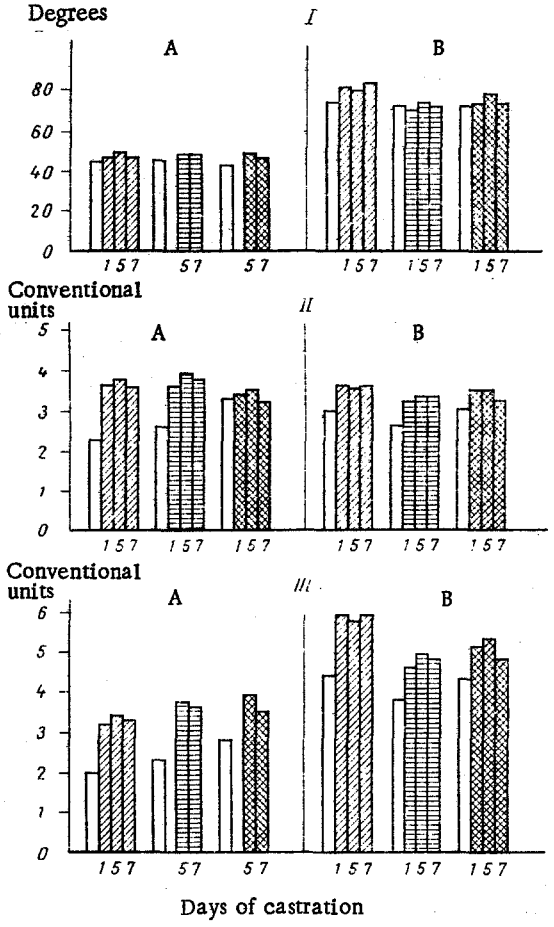

Fig. 1. Changes in protein content in neurons of anterior and mediobasal hypothalamus on neonatally castrated sexually mature male rats. I) Protein concentration (phase shift of beam of light in deg.); II) size (in conventional units); III) dry weight (in conventional units). A) Nucleus; B) cell. Unshaded columns — control; obliquely shaded columns — medial preoptic region; horizontally shaded column — arcuate nucleus; cross-hatched columns — ventromedial nucleus.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats. Males were castrated during the first week after birth. Intact animals of the same litter were used as the control. The animals were decapitated at the age of 83-85 days. The hypothalamic region was fixed with a mixture of basic formalin, 96° alcohol, and glacial acetic acid in the ratio of 3:1:0.3. The material was embedded in paraffin wax and serial sections were cut to a thickness of 5 μ.

Neurons of the medial part of the preoptic region and of the arcuate and ventromedial hypothalamic nuclei were investigated. In each series of experiments 3 or 4 animals were used, and 70-100 cells were measured from each animal. The difference in the course of rays of light passing through the object and through the medium in which the object was embedded (the phase shift) was measured by means of an interference microscope and the dry weight of the cell and nucelus was calculated by the equation:

$$m = \frac{\delta \cdot S}{100\rho},$$

where δ is the phase shift of the ray of light; S the area of the cell or nucleus; ρ the specific increase in refractive index, which is 0.0018 for protein [2]. The area of the cell was measured in conventional units by means of a special square attachment to the microscope ocular. The size of the nuclei was determined previously [1]. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The protein concentration and also the dimensions of the neurons in the anterior and mediobasal hypothalamus were increased in neonatally castrated sexually mature male rats (Fig. 1A). The dry weight of the neurons in the medial part of the preoptic region was increased on average by 34% ($P < 0.001$) and in the arcuate region of the hypothalamus by 25%

($P < 0.001$) in animals castrated on the first to seventh day after birth; the dry weight of the neurons in the ventromedial hypothalamic nucleus was increased on average by 22% ($P < 0.001$) in animals castrated on the 1st and 5th days after birth.

More definite changes were observed in the nuclei of these nurons (Fig. 1B). The protein concentration in the nuclei in the medial part of the preoptic region increased on average by 68% ($P < 0.001$) and in the nuclei of the arcuate region by 55% ($P < 0.001$) in animals castrated on the first to seventh day after birth. In the ventromedial region of the hypothalamus the dry weight of the nuclei increased by 39% ($P < 0.001$) in animals castrated on the fifth day of postnatal development. The protein concentration in the hypothalamic nuclei of animals castrated on the first day after birth was not measured. In animals castrated on the seventh day after birth the dry weight, and also the dimensions, of the neurons of the ventromedial hypothalamic nucleus showed a small increase (by 12%; $P < 0.001$), and no changes were found in the nuclei of these neurons.

After measuring the nuclei of neurons in the anterior and middle hypothalamus, Pfaff [12] also showed that the nuclei of neurons in the preoptic region are enlarged in males castrated on the seventh day after birth, but observed no changes in these parameters in the ventromedial hypothalamic nucleus. Neurons of the ventromedial nucleus of the hypothalamus in rats reach a characteristic size of sexually mature animals by the 10th day of postnatal life, whereas neurons of the arcuate and suprachiasmal nuclei increase in size up to the 20th day of life [9]. It can therefore by tentatively suggested that differentiation of the ventromedial nucleus is complete sooner than that of other regions of the hypothalamus.

The increase in dry weight of neurons in the preoptic region and mediobasal hypothalamus of neonatally castrated rats observed in this investigation may be evidence of intensified synthetic activity in neurons responsible for regulating both the cyclic and the tonic secretion of gonadotropic hormones in sexually mature animals. Data obtained by other workers in experiments with administration of labeled amino acids also support this hypothesis. Nakai [10] showed a selective increase in the uptake of leucine-^{3}H into protein of the arcuate nucleus in rats castrated on the 21st day of prenatal life. Meanwhile administration of testosterone [6] to newborn females reduced the incorporation of labeled amino acids into proteins of the anterior and mediobasal hypothalamus and also led to a decrease in synthesis of releasing factor, stimulating the secretion of luteinizing hormone, in the hypothalamus of sexually mature animals [8]. Considering the protein nature of the releasing factors regulating the secretion of gonadotropic hormones (GHRF) in mammals and also data indicating that the structures of the anterior and mediobasal hypothalamus participate in GHRF synthesis [15], it can only be suggested that the increased synthetic activity of the neurons in the hypothalamic regions studied is evidence that neonatal castration leads to increased intensity of metabolic processes connected with GHRF synthesis in sexually mature animals.

Sex hormones are known to exert their effect on hypothalamic neurons through interaction with a specific receptor protein. In the modern view, an essential step preceding the inducing action of androgens on the brain is their aromatization into estrogens [13]. Investigation of nuclear receptors of the neonatal hypothalamus [14] showed that in males aged 4 days the binding of estradiol-^{3}H is much lower than in females of the same age; castration of males on the second day after birth led to increased uptake and accumulation of estradiol-^{3}H by nuclei in animals aged 4 days. In the critical period of sexual differentiation of the brain, testicular hormones evidently exert their influence on estrogen-receptive proteins of the hypothalamus, making them less sensitive to estrogens, and thereby disturbing the cyclic mechanism of secretion of gonadotropins located in the preoptic region of the hypothalamus [4].

In the light of the above findings it can be postulated that the increase in size of the nuclei of neurons in the preoptic region and mediobasal hypothalamus up to that characteristic of females [1], observed in neonatally castrated males, and the correlating increase in dry weight of the neurons may be evidence that estrogen-binding receptors in males, in the absence of the inducing action of androgens, may be formed in the same way as in intact females, so that the character of sexual differentiation of the hypothalamus in neonatally castrated male rats is evidently determined according to the female type.

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