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EFFECT OF MALE SEX HORMONES ON SPECIFIC UPTAKE AND RELEASE OF ^{3}H-SEROTONIN BY THE RAT HYPOTHALAMUS IN VITRO

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UDC 616.831.41-008.94:577.175.823]-02:577.175.62]-092.9-07

KEY WORDS: serotonin uptake and release; in vitro perfusion; sexual differentiation of the brain; rat hypothalamus

In the perinatal ontogeny of the rat sex hormones play the role of inducers of cell differentiation in target cells. The main target region in the brain for these hormones is the hypothalamus [9], and possibly its serotoninergic system. Indirect proof of this may be given by data on sexual dimorphism in the serotonin (5-HT) content and the distribution of serotoninergic fibers in the sexual dimorphic nucleus of the medial preoptic region of the hypothalamus [12]. We showed previously that blocking the action of male sex hormones from the 1st day of postnatal life leads to an increase in the 5-HT content in the hypothalamus of sexually mature males to the level observed in females [1]. Sexual dimorphism in 5-HT metabolism begins to appear, evidently, during the "critical period" of sexual differentiation of the brain.

The aim of this investigation was to study the role of male sex hormones in differentiation of the serotoninergic system of the brain. Using a model with exclusion of the action of sex hormones in the period of sexual differentiation of the brain by castrating males from the 1st day of life, we studied the basic characteristics of functional activity of the serotoninergic elements of the hypothalamus, namely specific uptake and Ca^{2+}-dependent release of 5-HT.

EXPERIMENTAL METHOD

Experiments were carried out on three groups of Wistar rats aged 4-5 months: 1) intact males, 2) intact females in the diestrus stage (D1 and D2), 3) males castrated on the 1st day of life. Material was taken at 2-3 p.m. in the cold. The part of the brain removed included the medial preoptic region and the anterior hypothalamus. Specific uptake of 5-HT, spontaneous release of ^{3}H-5-HT and release evoked by K^+-depolarization, from fragments of the hypothalamus were studied by an "isotopic" method, which was described in detail by the writers previously [15]. To detect specific uptake in the control, unlabeled 5-HT was added to the incubation medium in a concentration of 10^{-5}

Laboratory of Hormonal Regulation, N. K. Kol'tsov Institute of Developmental Biology, Russian Academy of Medical Sciences, Moscow. (Presented by Academician of the Russian Academy of Medical Sciences A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 6, pp. 653-655, June, 1992. Original article submitted February 12, 1991.

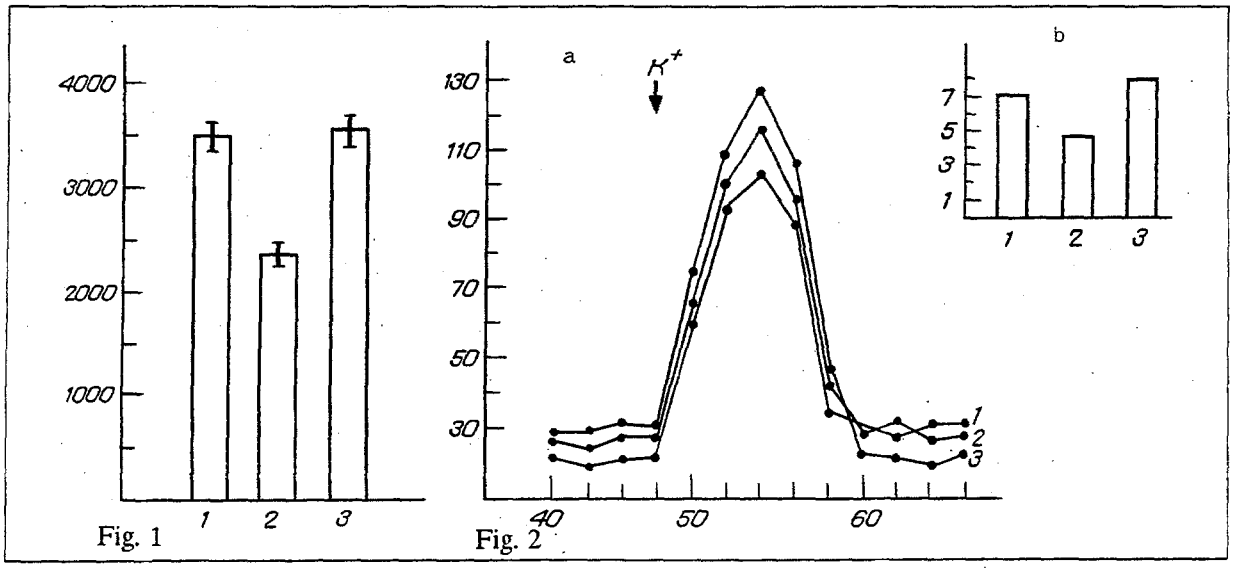

Fig. 1 Fig. 2

Fig. 1. Specific uptake of ^{3}H-5-HT by hypothalamus of female (1) and male (2) rats and by neonatally castrated male rats (3). Ordinate, radioactivity (in cpm/min/mg tissue).

Fig. 2. Spontaneous and K^+-stimulated release of ^{3}H-5-HT from fragments of hypothalamus; a) abscissa, time (in min); ordinate, radioactivity (in cpm/400 μl fraction/mg tissue of perfused fragment), b) ordinate, ratio of K^+-evoked release of ^{3}H-5-HT to spontaneous. Remainder of legend as to Fig. 1.

M. ^{3}H-5-HT was added to the medium in a concentration of $25 \cdot 10^{-9}$ M (specific activity 178-20 Em/mmole, "Amersham"). After incubation in medium with ^{3}H-5-HT, some fragments of the hypothalamus were perfused at the rate of 400 μl/min and 2-min fractions were collected in order to determine spontaneous and K^+-stimulated release of 5-HT.

EXPERIMENTAL RESULTS

The "isotopic" method of determining specific uptake of 5-HT by hypothalamic neurons enables the development of the network of serotoninergic fibers in the hypothalamus of males and females, and also in males in the absence of influence of sex hormones, to be judged indirectly. It was found that in sexually mature intact males specific uptake of ^{3}H-5-HT by the serotoninergic structures of the anterior hypothalamus is significantly lower than in females.

Castration of the animals on the 1st day of life led to an increase in specific uptake of 5-HT by sexually mature males up to the level observed in females (Fig. 1), and this may evidently be associated with an increase in the number of serotoninergic elements in the hypothalamus. The results are in good agreement with those of an immunohistochemical analysis of the distribution of serotoninergic structures in the anterior hypothalamus of rats, which showed that the number of serotoninergic fibers in the sexually dimorphic nucleus of the medial preoptic region of the hypothalamus in males is significantly less than in females [12]. Sexual dimorphism in the number of 5-HT-binding sites, found in the hypothalamus, is explained by some workers [3] by a change in the concentration of serotoninergic receptors. The possibility therefore cannot be ruled out that sex hormones have an influence on the membrane mechanisms of 5-HT transport.

In a study of the ability of the rat hypothalamus to secrete ^{3}H-5-HT taken up previously, we were unable to find any sex differences in the rate of its spontaneous release. However, the response to K^+-depolarization in the anterior hypothalamus of intact males was significantly lower than in females. In the hypothalamus of males castrated neonatally the amplitude of the response to the depolarizing agent rose to the level observed in females (Fig. 2).

Blocking the action of male sex hormones from the 1st day of postnatal life thus leads to increased uptake and release of 5-HT in the hypothalamus of mature males.

Considering data showing that injection of testosterone propionate into females in the perinatal period of development leads to a decrease in the number of serotoninergic elements in the anterior hypothalamus to the level observed in males, whereas castration of adult animals is not accompanied by any change in their number [13], it can be tentatively suggested that male sex hormones in the newborn have a morphogenetic action on the formation of the brain serotoninergic system.

The mechanism of the inducing action of sex hormones on brain differentiation is not yet sufficiently clear. We know that androgens can affect membrane permeability through induction of synthesis of membrane proteins concerned with ion transport. Since monoamine receptors are membrane proteins, it can be postulated that sex hormones are involved in their synthesis and degradation [4]. Even in newborn rats sex differences in the organization of the plasma membrane in hypothalamic neurons have been found: a larger number of exo-endocytotic outgrowths and protein particles has been found in females than in males on neuron membranes of the arcuate nucleus, obtained by the freeze-etching method, and at a later age, a larger number of synapses is found in females [11]. However, in females receiving testosterone propionate on the 5th day of life, the design of the membranes was the same as that characteristic of males [5]. Regulation of release of 5-HT is known to be effected through auto-, hetero-, and coreceptors [8]. A change in their number as a result of the inductive action of sex hormones can lead to disturbance of autoregulation of 5-HT synthesis, manifested as a change in its content in the hypothalamus and midbrain [6]. Disturbance of the molecular mechanisms of reception and transport of the hormonal signal, maintaining an adequate response of the cell to the stimulus, is sometimes accompanied by death of the cells [10]. Since the main source of serotoninergic fibers in the anterior hypothalamus is the neurons of the mesencephalic nuclei raphe, where cells accumulating sex hormones are found [14], it can be postulated that the midbrain is the target region for these hormones. However, the elucidation of this problem requires further research.

Thus male sex hormones in the newborn are involved in the mechanisms of sexual differentiation of the serotoninergic-system of the brain, and this is expressed as a change in the functional state of that system in adult animals.

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