# SEX HORMONE RECEPTORS IN THE HYPOTHALAMUS AND THEIR ROLE IN SEXUAL DIFFERENTIATION OF THE MALE RAT BRAIN

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The main events connected with sexual differentiation of the hypothalamus are known to take place in males under the influence of testicular hormones. Meanwhile much information is present in the literature on the effect not only of testosterone, but also of estradiol, on differentiation of the CNS [7]. As a result of the action of these hormones the male hypothalamus loses its ability to control the gonadotropic function of the pituitary in the manner of the female, which possesses a hypothalamus of the type found in animals of both sexes on the eve of the "critical" period. We also know that in males, just as in females both estradiol and testosterone are present in the blood [1]. Furthermore, the hypothalamus and limbic brain of sexually mature and neonatal male rats contain enzymes which aromatize androgens into estrogens [11], and there must be some biological meaning for this. The fact that the highest activity of aromatization is observed at the "critical" period [13] evidently indicates the importance of this process in differentiation of the male rat brain and the necessity for the presence of both sex hormones and, consequently, of receptors for them, for its implementation. The question accordingly arises of the presence of such receptors in the CNS and their characteristics in the period of neonatal development of male rats.

In this investigation changes in the level of receptors for sex hormones in the hypothalamus and cerebral cortex of male rats were studied on the 1st through the 5th days of postnatal life, i.e., in the period of sexual differentiation of the CNS, and the results obtained were compared with the levels of luteinizing hormone (LH) and sex hormones in the peripheral blood in order to discover any correlation between these parameters.

## EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats aged 1, 3, and 5 days. The hypothalamus and cerebral cortex from 25 animals were homogenized in buffer (pH 7.4) containing 0.01 M Tris-HCl and 0.0015 M EDTA, and centrifuged for 10 min at 800g. The supernatant was separated and the nuclei were further purified by Chauveau's method in the modification of Kedrova and Orlova [2]. To obtain the cytosol fraction, the supernatant was centrifuged for 1.5 h at 105,000g, on a Spinco 65-L2 ultracentrifuge. 2,4,6,7-$^3$H-Estradiol-17$\beta$ ($^3$H-$E_2$) and 1,2,6,7 $^3$H-testosterone ($^3$H-T) with specific activity of 93-105 Ci/mmole, were used as labeled hormones. Binding sites of estradiol and testosterone (Nc) and the association constant ($K_{ass}$) in the cytosol fraction were determined by the method described previously [3]. The number and the association constant of the nuclear receptors were determined by the method in [4].

## EXPERIMENTAL RESULTS

As will be clear from Table 1, the concentration of cytoplasmic binding sites for estradiol and testosterone was highest on the 1st day after birth and fell until the 5th day of postnatal development ($P < 0.01$).

The same time course of changes in the concentration of hormone-binding molecules also was observed in the nuclear fraction.

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TABLE 1. Values of Association Constant ($K_{ass}$) and Concentration of Specific Binding Sites (Nc) for Estradiol and Testosterone in Hypothalamus and Cerebral Cortex of Male Rats during Neonatal Development (M ± m)

| Tissue | Age of animals, days | Receptors for estradiol | | | | Receptors for testosterone | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | cytosol | | nuclear fraction | | cytosol | | nuclear fraction | |
| | | Nc, moles · $10^{-13}$/mg protein | $K_{ass}$, $N^{-1} \cdot 10^{10}$ | Nc, moles · $10^{-11}$/mg protein | $K_{ass}$, $N^{-1} \cdot 10^{9}$ | Nc, moles · $10^{-13}$/mg protein | $K_{ass}$, $N^{-1} \cdot 10^{10}$ | Nc, moles · $10^{-11}$/mg protein | $K_{ass}$, $N^{-1} \cdot 10^{9}$ |
| Hypothalamus | 1 | 43,3±2,1 | 1,2±0,3 | 18,9±2,0 | 1,4±0,4 | 17,5±0,6 | 1,2±0,3 | 5,9±1,3 | 2,9±0,4 |
| | 3 | 25,1±2,6* | 0,5±0,1 | 8,5±1,8** | 1,1±0,3 | 13,2±4,2 | 0,9±0,1 | 3,3±0,5* | 1,6±0,2 |
| | 5 | 16,6±5,6* | 0,8±0,2 | 8,2±0,6 | 1,0±0,1 | 9,3±0,3* | 1,0±0,2 | 2,3±0,4* | 1,9±0,2 |
| Cortex | 1 | 10,5±3,5 | 1,1±0,3 | 8,1±1,0 | 2,8±0,5 | 13,0±2,5 | 1,0±0,3 | 3,1±0,9 | 2,1±0,4 |
| | 3 | 19,0±2,0 | 1,0±0,2 | 4,1±0,4 | 2,2±0,1 | 10,6±3,3 | 0,9±0,3 | 2,1±0,5 | 1,9±0,1 |
| | 5 | 14,5±3,1 | 0,8±0,1 | 3,2±0,2 | 3,6±0,6 | 6,1±0,3 | 1,1±0,2 | 1,3±9,2 | 2,2±0,3 |

Legend. Number of experiments was 5-7. *P < 0.01 for comparison of rats aged 3 and 5 days with rats aged 1 day, **P < 0.05 for comparison of rats aged 3 days and 1 day.

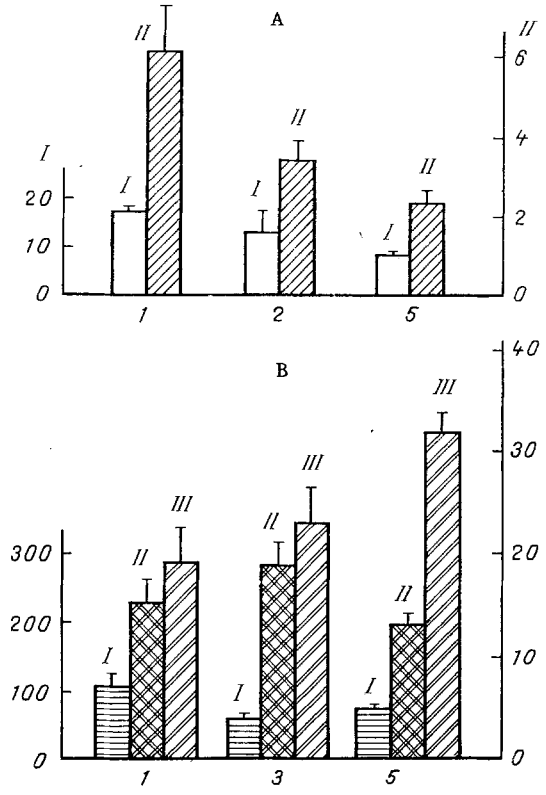


Fig. 1. Concentration of cytoplasmic and nuclear receptors for estradiol in hypothalamus (A) and blood levels of estradiol, testosterone, and LH (B) of male rats during postnatal development. Abscissa, animals' age (in days); ordinate: A) bound $^3H$-$E_2$: $I_o$ in cytosol of hypothalamus (in moles/mg protein$\cdot 10^{-13}$), II) in nuclear fraction (in moles/mg DNA$\cdot 10^{-11}$): B: I, II) $E_2$ and T, respectively (in pg/ml), III) concentration (in mg/ml) in blood plasma.

Specific macromolecules possessed a high degree of affinity for estradiol or testosterone, for their association constant ($K_{ass}$) was high, and lay between $0.5 \cdot 10^{10}$ $M^{-1}$ and $1.2 \cdot 10^{10}$ $M^{-1}$ for the cytoplasmic fraction and between $1.0 \cdot 10^{9}$ $M^{-1}$ and $2.9 \cdot 10^{9}$ $M^{-1}$ for the nuclear fraction of the hypothalamus.

To study sex hormone receptors in the CNS of the male rats, the parameters of binding of these hormones were determined in the cerebral cortex also. As a result of these investiga-

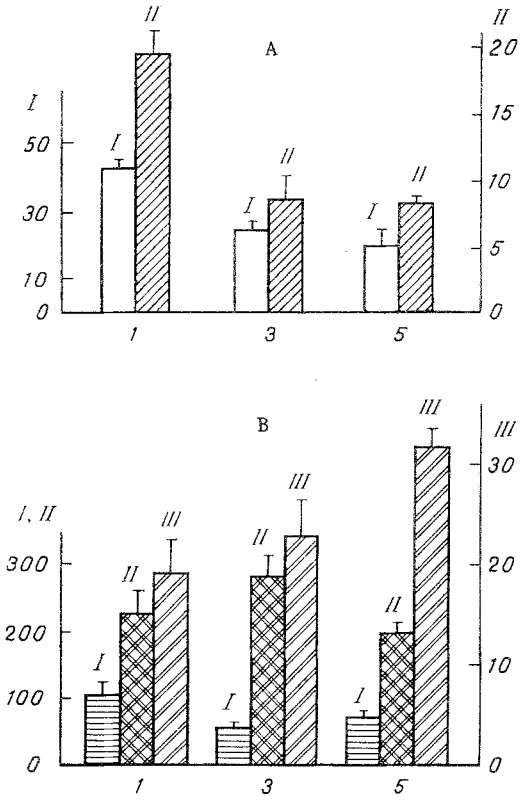


Fig. 2. Concentration of cytoplasmic and nuclear receptors for testosterone in the hypothalamus (A) and blood levels of estradiol, testosterone, and LH (B) in male rats during postnatal development. Abscissa, age of animals (in days); ordinate: A) Bound $^3$H-T: I) in cytosol of hypothalamus (in moles/mg protein• $10^{-13}$), II) in nuclear fraction (in moles/mg DNA•$10^{-11}$); B: I, II) concentration of $E_2$ and T, respectively (in pg/ml), III) LH concentration (in mg/ml) in blood plasma.

tions, correlation was found between the concentration of receptors for the hormones and the animals' age, as in the hypothalamus (Table 1), although the absolute number of receptor sites was somewhat less, in both the cytoplasm and the nuclear fraction, in the cortex than in the hypothalamus. The fact that the concentration of sex hormone receptors in the male rat cortex fell toward the end of the "critical" period evidently indicates that these receptors play an important role, together with hypothalamic receptors, in sexual differentiation of the brain.

There is evidence in the literature that estradiol ($E_2$) whose biological effect cannot be realized without the participation of specific receptor proteins, participates in establishment of the function of cortical neurons, and disappearance of receptors from cortical cells coincides with the end of differentiation of neurons and cessation of growth and myelinization of axons [6, 9].

Investigation of the ability of different unlabeled hormones to replace labeled estradiol or testosterone competitively showed that unlabeled estradiol considerably reduced (by 40-55%) binding of $^3$H-$E_2$ in both the cytoplasmic and the nuclear fractions of the hypothalamus, whereas unlabeled diethylstilbestrol (DES) in the cytosol reduced binding of $^3$H-$E_2$ by only 8-26%; the greatest competition for binding sites, moreover, was observed on the 5th day of life. In the nuclear fraction, on the other hand, affinity of binding of proteins to DES was greater, and was close to that for $E_2$ (35%). Testosterone competed weakly for binding sites with $^3$H-$E_2$ in both the cytosol and the nuclear fraction.

When these data are analyzed it must be noted that the hypothalamic tissue of neonatal rats, both male and female, contains α-ketoprotein, which evidently does not allow receptors for $E_2$ in the cytosol of the male hypothalamus to be detected. Data in the literature, however, indicate that such receptors are present [5, 8]. In view of the results, it can be postulated that cytoplasmic receptors for $E_2$ are present in the male rat hypothalamus, and that their number increases during the first 5 days after birth.

There is also evidence in the literature that receptors for $E_2$ are present in male rats in the neonatal period in the nuclear fraction of the hypothalamus [10-12]. When investigating the ability of the receptors to bind testosterone specifically, the writers showed that dihydrotestosterone and testosterone compete equally well for binding sites both in the cytosol (35-54%) and in the nuclear fraction (41-55%). It will be noted that estradiol displaced hardly any $^3$H-T from its complex with the receptor in the cytosol (5-25%), whereas in the nuclear fraction it was highly effective (40-50%). Only corticosterone was ineffective in this respect.

To determine the role of sex hormone receptors in the hypothalamus of male rats in the neonatal period, it is evidently necessary to compare the concentrations of sex hormones and LH in the blood with the concentrations of receptors in the nuclear fraction of the hypothalamus during this age period. As will be clear from Figs. 1 and 2, the estradiol concentration was highest on the 1st day after birth (107.4 pg/ml), after which it fell to 55.7 pg/ml on the 3rd day ($P < 0.05$) and it increased to 72.6 pg/ml on the 5th day. A different picture was observed with the testosterone concentration. This rose until the 3rd day (from 225.5 ± 35.0 pg/ml on the 1st day to 280.7 ± 38.3 pg/ml on the 3rd day, and fell until the 5th day after birth (198.5 ± 15.4 pg/ml; $P < 0.05$). The LH level rose under these circumstances. Comparison of these data with the concentration of receptors for estradiol and testosterone (Figs. 1 and 2) showed negative correlation between the concentration of the hormone with their receptors and the LH level; this suggests that negative feedback may take place in the hypothalamo-hypophyseo-gonadal system as early as during neonatal development of male rats.

Thus, in male rats on the 1st day after birth receptors for estradiol and testosterone are present, and they enable the action both of the testicular hormone itself, and of that of estradiol, formed locally in the process of aromatization, on intracellular processes that constitute the essence of sexual differentiation of the brain, to be realized.

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