Effect of Gonadectomy on Activity of Neuronal 3β-Hydroxysteroid Dehydrogenase in Some Brain Structures

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 5, pp. 748-750, May, 2012
Original article submitted March 23, 2011

3β-Hydroxysteroid dehydrogenase is a key enzyme in the synthesis of steroid hormones in steroid-producing organs, including the brain producing neurosteroids. 3β-Hydroxysteroid dehydrogenase activity can be a marker of steroid-producing cells. We present the results of histochemical assay of this enzyme in the neocortex, hippocampus, and cerebellar cortex of gonadectomized prepubertal rats. The positive reaction was detected in hippocampal neurons, ganglionic layer cells of the cerebellar cortex (Purkinje cells), and solitary neocortical neurons of male and female rats. Gonadectomy significantly increased enzyme activity in neocortical (layer V) and hippocampal neurons and had no effect on the intensity of the reaction in Purkinje cells.

Key Words: *3β-hydroxysteroid dehydrogenase; neocortical, hippocampal, and cerebellar neurons; neurosteroids*

The brain is a target organ for steroid hormones [1,2-4,6,9]. They determine gender differences of the organ during the early ontogeny and modulate behavioral reactions in male and female animals [3,5]. Administration of testosterone derivatives, mineralcorticoids, and glucocorticoids to pregnant rats as well as hemiovariectomy and stress exposures modulate morphometric and histochemical parameters of the brain in their progeny [4].

At the same time, the brain normally functions against the background of highly variable levels of steroid hormones in the organism and this variability increases under pathological conditions. For instance, the blood concentration of sex hormones normally changes by several times over the menstrual cycle, during growth and sexual maturation, and at menopause [3]. This suggests the existence of mechanisms "neutralizing" the effects of considerable fluctuation of steroid concentrations acting on the brain. These mechanisms are probably related to the steroid-producing capacity of neurons and glial cells [6,11]. Neurosteroids are synthesized in different structures of the brain, in particular in neurons of the hippocampus and neocortex. Maximum intensity of neurosteroid synthesis was reported in Purkinje cells [7-9,11]. It can be hypothesized that the mechanisms of neutralization include modulation of neurosteroid synthesis aimed at damping the fluctuations of steroid concentrations in the brain. This hypothesis can be verified by histochemical analysis of 3β-hydroxysteroid dehydrogenase (HSD) activity in these cases. This enzyme catalyzes the formation of progesterone, a neurosteroid regulating various brain functions and a precursor of brain estrogens, androgens, and corticosteroids [3,6-8].

Here we performed a histochemical analysis of neuronal HSD activity in some structures of gonadectomized rats.

MATERIALS AND METHODS

Prepubertal rats (at the age of 30 days) were subjected to gonadectomy or sham-operation under ether nar-

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0007-4888/12/1535-0784

cosis. Gonadectomized (6 males and 4 females) and control rats (6 males and 5 females) were taken from the same litters and did not differ by body weight at the age of 30 days (Table 1); the animals were kept in a vivarium and received water and food *ad libitum*. At the age of 42 days the animals were decapitated, weighed, the brain was removed, and the cerebellum and the parietal lobe of the cortex were isolated. The cerebellum and the parietal lobe were placed in separate blocks for obtaining cryostat section perpendicular to the long axis of the brain (4-5 sections, 40 μ) on a Leica CM 1850 cryostat. The sections were mounted on coverslips and covered with an incubation solution for HSD detection containing dehydroepiandrosterone (substrate), NAD, and NBT (all reagents were from Sigma). The reaction was carried out in a thermostat at 37°C for 30 min. The preparations were embedded in glycerin-gelatin and Canadian balm.

The sections were examined under an Olimpus microscope. The intensity of the reaction was measured on preparations embedded in glycerin-gelatin on a Mekos device at λ=550 nm in the cytoplasm of parietal cortex layer V and hippocampal field I neurons and cerebellar Purkinje cells. In addition, the number of positive Purkinje cells on the cerebellar gyrus surface per standard section length (500 μ) was determined.

RESULTS

In the control group, the number of HSD-positive cells in the neurocortex was low, because most of them were not stained during the reaction. The positive reaction was noted in few nerve cells in layers V and VI lying separately or arranged in groups of 2-3 cells. In positive cells, the reaction products (violet formazan granules) were seen in both perikaryons and processes

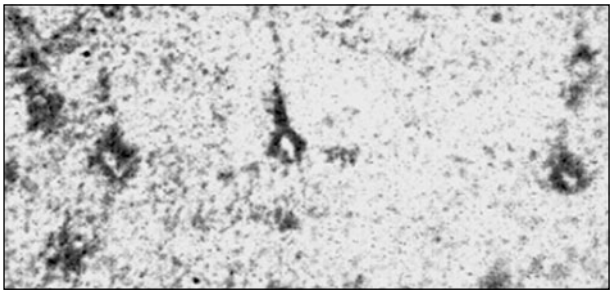

Fig. 1. Neocortex, layer V of the anterior parietal lobe Reaction for HSD, ×900.

(Fig. 1). In the hippocampus, the positive reaction was detected in all, or at least in the great part of neurons (Fig. 2). The reaction products were detected in the soma and processes of neurons. In the cerebral cortex, the intensive reaction was observed in Purkinje cells, while granular layer neurons were not stained (Fig. 3).

Cytophotometry revealed no significant differences in the reaction intensity between males and females and showed that in Purkinje cells this parameter was 2-fold higher than in other studied neurons (Table 1). These findings agree with the data on high intensity of neurosteroid synthesis in Purkinje cells obtained by other methods [8-10].

Analysis of the brain in experimental rats showed that gonadectomy had no effect on brain weight in animals of both sexes, which probably attests to insignificant role of sex steroids in the regulation of brain growth in rats during the specified age interval. The number of Purkinje cells per standard gyrus length of the cerebral cortex and reaction intensity in these cells were similar in castrated and control animals (Table 1). At the same time, HSD activity in neocortical layer V neurons in experimental males was higher than in controls by 43.6% and in females by 27.7% and in hippocampal neurons

TABLE 1. HSD Activity in Brain Neurons of Gonadectomized Rats (*M*±*m*)

Parameter	Males		Females	
	control (*n*=6)	experiment (*n*=6)	control (*n*=5)	experiment (*n*=4)
Body weight, 30 days, g	67±2	66.0±3.2	65.0±5.3	67.0±7.7
Body weight, 42 days, g	128.0±2.5	109.0±4.3*	128.0±6.9	140.0±8.1
Brain weight, mg	1673±14	1665±27	1611±27	1605±37
HSD activity, arb. units				
Neocortical layer V neurons	0.339±0.039	0.487±0.043*	0.258±0.021	0.345±0.026*
Hippocampal neurons	0.332±0.038	0.424±0.02*	0.369±0.023	0.427±0.016*
Purkinje cells	0.717±0.037	0.781±0.059	0.758±0.019	0.836±0.093
Number of Purkinje cells per 500 μ	16.1±0.4	16.3±1.4	16.4±0.4	16.0±0.4

Note. *$p<0.05$ in comparison with the control.

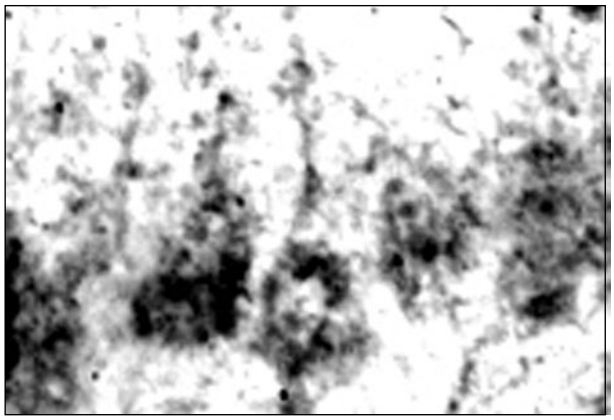

Fig. 2. Hippocampus. Reaction for HSD, ×900.

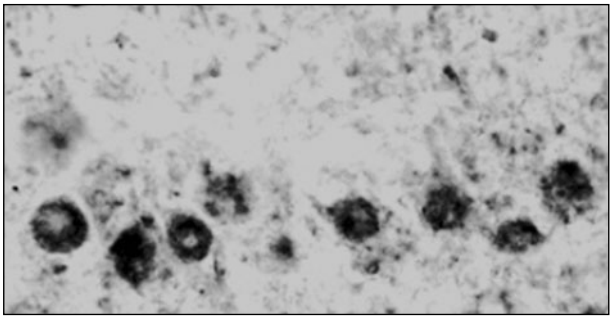

Fig. 3. Cerebellar cortex. High staining intensity in Purkinje cells, no reaction in the granular layer, ×900.

by 27.7 and 15.7%, respectively. These differences between the groups were significant (Table 1).

Thus, analysis of preparations and cytospectrophotometry revealed no gender differences in the location of HSD-positive neurons and the intensity of the reaction in both the control and gonadectomized rats. This is probably related to the fact that HSD catalyzes the synthesis of progesterone, a neurohormone and a precursor of various steroid hormones in the brain (estrogens, androgens, corticosteroids) that were not analyzed in this study. When analyzing the obtained results we should take into account the fact that neurosteroids are synthesized in certain neurons and the intensity of this process can vary [1,6,9,10]. For instance, the prenatal stress can modulate the intensity of synthesis of these compounds [2].

Our findings confirm the possibility of intensification of neurosteroid synthesis in the brain upon a decrease in blood concentration of hormones produced in the gonads or adrenals. Changes in qualitative composition of the neurosteroids synthesized under these conditions require further investigation.

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