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DEPENDENCE OF ANDROGENIZATION ON DIFFERENTIATION OF THE HYPOTHALAMIC CENTERS

I. I. Dedov and N. A. Demina

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Dependence of the sterilizing action of androgens on the level of differentiation of the hypothalamic centers in the postnatal period of development was studied in female rats. Asynchronous development of the arcuate nucleus (AN; the tonic center) and the suprachiasmatic nucleus (SCN; the cyclic center) was found. Neurons of AN begin to produce granules of secretion in 20-day embryos. The first neurons with granules of secretion are found in SCN in rats aged 5-7 days. Injection of testosterone propionate induces an anovulatory cycle in females during the first 7 days after birth, on account of inhibition of development of the hypothalamic cyclic center.

KEY WORDS: hypothalamus; anovulatory cycle; arcuate nucleus; suprachiasmatic nucleus.

In female rats the 4-5-day ovulatory cycle is determined by the tonic and cyclic centers of the hypothalamus. The tonic center regulates the development of the follicles in the ovaries between ovulations. The cyclic center is connected to the neuroendocrine system only in the period of ovulation, i.e., it maintains the "ovulatory release" of luteinizing hormone essential for rupture of the ripe follicle and formation of the corpus luteum [3]. The tonic center is located in the zone of the mediobasal hypothalamus, including the arcuate nucleus (AN). Among the structures of the cyclic center, the suprachiasmatic nucleus (SCN) of the anterior hypothalamus plays a definite role [1, 4, 5].

Disturbance of the function of the cyclic center leads to the establishment of an anovulatory cycle in females with the development of persistent follicles and follicular cysts in the ovaries. The latter become the source of formation of a high level of estrogens, which play the basic role in the pathogenesis of dyshormonal tumors.

Neonatally androgenized female rats are a widely used experimental model of the anovulatory cycle in neuroendocrinology. Characteristically an anovulatory cycle can be induced by androgens only in the first days after birth of the females.

The object of the present investigation was to examine whether the action of androgens on the formation of the hypothalamic-hypophyseal-gonadal system in females is dependent on the time of differentiation of the tonic and cycle centers of the hypothalamus and their incorporation into the general neuroendocrine system.

EXPERIMENTAL METHOD

Experiments were carried out on female Wistar rats taken on the 17th and 20th days of embryonic development and 1, 3, 5, 7, 10, 15, 30, 45, and 60 days after birth. For histological examination the hypothalamus was fixed in Bouin's solution and 10% neutral formalin, and then embedded in paraffin wax. Serial sections cut to a thickness of 7 μ were stained with paraldehyde–fuchsin and gallocyanin. To determine the dynamics of development of AN and SCN neurons, the axes of the nucleus and cytoplasm of 200 cells were measured with an ocular micrometer. The volume of the nucleus and cell was calculated by the equation:

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TABLE 1. Dimensions of Nuclei and Cytoplasm (in μ^3) of AN and SCN Neurons in Female Rats during Postnatal Period (M ± m)

Hypothalamic nuclei		Age of female rats, days					
		1	7	14	21	30	90
AN	Volume of nucleus	181±10	217±14	212±16	228±18	296±21*	308±28*
	Volume of cytoplasm	168±12	192±13	272±18*	483±27†	531±46†	576±41*
SCN	Volume of nucleus	142±12	156±7	194±6*	188±10	246±14†	248±12†
	Volume of cytoplasm	122±8	148±18	185±16	312±26	446±32†	428±36†

*P < 0.05 compared with animals of previous age.
†P < 0.001.

TABLE 2. Androgenization of Female Rats after a Single Injection of 1250 μg Testosterone Propionate at Different Times of Postnatal Development

Age of female rats, days	Total number	% of sterile rats
1	25	100
3	17	100
5	22	100
7	20	66
10	15	0
15	18	0

$$V = {}^3/_4\pi \left(\frac{l_1 \cdot l_2}{2}\right),$$

where l_1 is the long axis and l_2 the short axis of the nucleus or the whole cell. The volume of the cytoplasm was determined by the equation $V_c = V_{neu} - V_{nuc}$, where V_{neu} is the volume of the neuron and V_{nuc} the volume of the nucleus.

For electron-microscopic investigation of AN and SCN the hypothalamus was fixed in a 2.5% solution of glutaraldehyde and postfixed in a 1% solution of osmium tetroxide. The material was embedded in araldite. Sections were stained with uranyl acetate and lead citrate and examined in the JEM-5 or JEM-7 electron microscope.

An oily solution of testosterone propionate in a dose of 1250 μg per animal was injected subcutaneously into the females at the age of 1, 3, 5, 7, 10, and 15 days. The androgenization phenomenon, i.e., permanent estrogen, was demonstrated in females at the age of 90 and 150 days on the basis of vaginal smears. At autopsy the ovaries were removed from the animals for histological examination. By means of a radioimmunological method, using the kits obtained from the firm CEA IRE SORIN, the estradiol concentration was determined in the blood plasma of androgenized and control animals.

EXPERIMENTAL RESULTS

Data on the dynamics of the nucleo-cytoplasmic ratios in neurons of AN and SCN in animals of different ages are given in Table 1. Characteristically the volume of the nuclei in AN neurons increased more rapidly than in SCN neurons, and in rats aged 7 days it reached a level which remained virtually unchanged during the next two weeks of postnatal period. In rats aged 30 days, judging from the size of the nucleus and cytoplasm, differentiation of the AN neuron was complete. As regards SCN, the increase in volume of the karyoplasm of these neurons continued during the first two weeks after birth, when the first peak was reached. The second peak was observed in the animals at the age of 30 days, when the volume of the cell nuclei and cytoplasm was equal to their volume in sexually mature (90 days) rats. The forced increase in volume of the cytoplasm in neurons of both hypothalamic nuclei began after stabilization of the size of the nuclei.

The course of differentiation of the AN and SCN neurons could be followed at the ultrastructural level by electron microscopy. The inducing influence of the nucleus and of the outer nuclear membrane on organelle formation in AN and SCN neurons was revealed. The hypertrophied nucleoli were the first of intensive formation of RNA particles which were concentrated in the zone of the nuclear pores. The number of free polysomes increased synchronously in the cytoplasm. The outer nuclear membrane plays the role of primary source of

formation of the endoplasmic reticulum. Differentiation of neurons was characterized by a progressive increase in the total area of the tubules of the granular cytoplasmic reticulum, an increase in the number and size of the mitochondria and the formation of cholinergic and monoaminergic synapses on the bodies and processes of the cells.

Since AN and SCN neurons produce (LH/FSH)-releasing hormones of peptide nature, a topic of special interest was the development of the apparatus in which the formation of the product is finalized in the form of granules, a characteristic feature of all glandular cells producing secretion for export. It was proposed to establish the beginning of their specific secretory function on the basis of the presence of granules of secretion in the Golgi apparatus of the differentiating AN and SCN neurons. Neurons with solitary granules of secretion were found in AN in 20-day embryos. After birth the number of cells forming secretory material increased rapidly and, at the same time, the process of secretion formation in them was activated. Among the population of SCN cells neurons with granules of secretion first appeared in rats aged 7 days. AN and SCN neurons starting to secrete had not yet reached a high level of differentiation, but synapses of different types were virtually always found on their bodies.

The results of androgenization of female rats of different ages are given in Table 2.

An anovulatory cycle with permanent estrus was discovered in the animals on the basis of vaginal smears. One injection of testosterone propionate produced a 100% effect in the rats during the first 5 days after birth. Among the animals receiving the androgen at the age of 7 days only 68% were sterile, and in females aged 10 and 15 days the hormone was ineffective. When these animals reached sexual maturity, they developed the normal cycle and became pregnant. In the ovaries of the females with an anovulatory cycle, histological examination revealed persistent follicles but no corpora lutea. The estrogen level in the blood plasma of the sterile rats (76.4 ± 3.2 pg) was significantly ($P < 0.01$) higher than that of the intact rats (48.6 ± 2.4 pg).

The results described above point to a causal connection between the development of the hypothalamic centers and induction of an anovulatory cycle in the female rats by androgens. In fact, if the tonic and cyclic centers are identified with AN and SCN respectively, it becomes evident that the tonic center is formed a considerable time before the development of the cyclic center. Female rats are born with a sufficiently well developed tonic center and some neurons of AN are already producing their specific secretion. As regards the cyclic center, neurons with granules of secretion were first found in SCN in females only after the age of 5 to 7 days. Japanese workers, choosing the dimensions of the nuclei of neurons of 8 hypothalamic nuclei of the anterior and middle hypothalamus as the criterion of the degree of differentiation, have recently also found that AN develops sooner than SCN and the other nuclei [2].

Sexual differentiation of the hypothalamus of the female type, i.e., differentiation of a cyclic center, is possible only in the absence of male sex hormone from the body. Androgenization of females during the first days after birth is essentially blocking the development of the cyclic center, for the tonic center is already functioning in these females. Follicles ripen in the ovaries but ovulation does not take place, for it is the cyclic center which is responsible for ovulation. The critical period in the development of female rats, before which exogenous hormones can disturb the formation of the female reproductive system, coincides with the time of development of the hypothalamic cyclic center.

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