Effect of Experimental Hyperprolactinemia on the Sexual Cycle and Folliculogenesis

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It is found that hyperprolactinemia results in the development of stable anovulation, in which the disruption of folliculogenesis sets in as early as at the stage of late preantral (graafian) follicles and manifests itself in a decrease of the mitotic activity in cells of the membrana granulosa. The concentration of follicle-stimulating hormone thereupon changes to different extents or remains at the control level. A direct influence of prolactin on intrafollicular estrogen production and on cell division is postulated.

Key Words: *ovary; follicle; prolactin; anovulation*

The treatment of hyperprolactinemic states is a high-priority issue in gynecology and endocrinology due to the low efficacy of treatment and the broad diversity of this gynecological pathology. The difficulties in tackling this problem in a narrow clinical setting spurred attempts to elaborate experimental models of hyperprolactinemic states using biological objects.

The aim of the present investigation was to study folliculogenesis and the nature of the estrous cycle in experimental hyperprolactinemia.

MATERIALS AND METHODS

The study was performed on ovaries of adult nonpedigree albino rats showing a stable 4-day estrous cycle. Hyperprolactinemia was induced by repeated injections of perphenazine in a single dose of 0.5 mg/kg [5], which selectively increases the release into the blood of an isoform of prolactin mitogenic for mammary gland cells. The preparation was administered once a day i.p. during 8 days (two consecutive estrous cycles). The changes in the nature of the estrous cycle were judged from vaginal smears studied twice a day. Intact pubertal rats with a stable 4-day cycle were the controls. Autopsy and histological examination of ovaries were performed on the 4th day of prolonged diestrus in experimental animals (ovaries of intact rats on the 2nd day of diestrus were the control). For general histological examination ovaries were routinely processed and serial 5 μ sections were studied. Morphometry of ovarian follicle pools was performed according to the Lintern-Moor classification [4] devised for rats. Follicles with a maximal section of oocytes (these oocytes contained nucleoli) were selected for morphometry in order to avoid mistakes and replicates. The mitotic index (MI) for cells of the membrana granulosa was calculated after Hirshfield [2,3]. The concentration of follicle-stimulating hormone was measured in peripheral blood plasma by radioimmune assay using CIS kits (France).

RESULTS

The pattern of variations in the estrous cycle of the experimental animals was as follows: 10 out of 11 rats injected with perphenazine from proestrus showed a prolongation of the transition to estrus, followed by a long permanent diestrus; 5 of the 6

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TABLE 1. Indexes of Ovarian Follicle Pool Volumes in Control and Experimental Animals

Experimental conditions	Pool of follicles						
	primordial	primary	growing	late preantral	medium antral	large antral	follicular cysts
Control, 2nd day of diestrus	1313.0±26.3	205.4±9.4	115.0±6.4	135.7±3.5	50.0±4.6	7.4±1.2	–
Perphenazine administration, 4th day of prolonged diestrus	1300.4±12.5	305.4±8.3	95.7±3.6	165.2±8.9	20.0±2.0	4.0±0.3	2–3 cysts per ovary

Note. Significance of differences $p<0.05$.

animals which began to receive the drug at estrus went over to permanent diestrus as well. All 25 rats which received the first injection of perphenazine at the beginning of diestrus preserved the picture of this phase on vaginal smears during several cycles.

When experimental and control follicle pool volumes at the gonadotropin-dependent stages of development (Table. 1) were compared, the changes of their ratios were clearly revealed under the experimental conditions and were as follows: against the background of an elevated number of follicles at the late preantral stages of development (from 135.7±3.5 in the control to 165.2±8.9 in the experiment, $p<0.05$) there was a reliable decrease in the number of medium-size (from 50.0±4.6 to 20.0±2.0, $p<0.05$) and large (from 7.4±1.2 to 4.0±0.3, $p<0.05$) antral follicles. In view of the fact that the decrease in the number of medium-size and large antral follicles occurred against the background of an enlarged late preantral follicle pool, it may be assumed that it is in this pool that certain changes arise under hyperprolactinemia to prevent the transition to the subsequent stages of development. Since the antral stages of folliculogenesis are strictly gonadotropin-dependent and this dependence is realized at the receptor level the number of receptors being directly proportional to the number of granulosa cells and to the level of their proliferative activity, we calculated the MI of the granulosa cells of follicles at the late preantral stages. In the control group the MI varied from 6.5 to 11.2% in different cases. This fact alone attested to a certain asynchronicity of the development of preantral follicles under physiological conditions, which is evidently basic to the selection of antral follicles. The value of the MI in the experiment (4.5-7.0%) testified to the substantial inhibition of granulosa proliferation in late preantral follicles under conditions of hyperprolactinemia.

The effect of hyperprolactinemia on the gonadotropin-dependent stages of follicle growth may be realized via its influence on the gonadotropin system and above all on the follicle-stimulating hormone (FSH), which is known to be a natural mitogen for granulosa cells. The endogenous FSH level varied in the plasma of hyperprolactinemic rats as follows (Table 2): it was below the control in 9 animals, above the control in another 9 rats, and did not differ from the control in 7 animals. The proliferative activity of granulosa cells was reliably decreased in the majority of cases, no matter how the endogenous FSH level changed. When hyperprolactinemic states were accompanied by a drop of the FSH level (they might even have caused it), the decline in proliferative activity might have occurred directly due to the deficiency of this gonadotropin. But how does the reduction in the level of proliferative activity mesh with normal or elevated FSH titers? Considering that such a powerful physiological effect of prolactin as inhibition of androgen aromatization has been demonstrated *in vitro,* one cannot rule out that the drop of the level of mitotic activity of granulosa cells in follicles at the late preantral stages may stem from a depressed intrafollicular production of estrogens, which are also natural mitogens of granulosa cells.

TABLE 2. Content of FSH in Plasma of Peripheral Blood in Rats during Period of Physiological and Prolonged Diestrus Induced with Perphenazine

Experimental conditions	Plasma concentration of FSH, μg/liter
Control, 2nd day of physiological diestrus (11)	104.0; 109.1; 111.8; 99.9; 105.7; 113.2; 116.6; 102.8; 109.9; 120.4; 109.0
Perphenazine administration, 4th day of prolonged diestrus (25)	70.2; 69.9; 64.4; 86.9; 58.2; 90.45; 75.4; 90.4; 80.0 (9) 111.0; 114.5; 108.1; 113.1; 111.5; 114.5; 112.3 (7); 125.3; 149.4; 144.4; 193.8; 120.0; 156.7; 123.7; 159.0; 154.0 (9)

Note. The number of animals is given in parentheses.

The diminished proliferation of granulosa cells in follicles at late preantral stages probably leads subsequently to a decrease of the mean number of granulosa cells in antral and preovulatory follicles and in a disruption of the physiology of the cell-cell relationships between granulosa and theca follicles. This manifests itself, in our opinion, in the cystoatresic transformation of a number of antral follicles observed in slides of ovaries of experimental animals against the background of a diminished population of "healthy" antral follicles (Table. 1).

Thus, hyperprolactinemia induced experimentally disrupts the physiological estrous cycle and causes anovulation in virtually 100% of cases.

The presumed onset of folliculogenesis "destruction" under hyperprolactinemic conditions occurs in the late preantral stages of development of the follicles, in whose membrana granulosa the mitotic activity of cells drops when the prolactin concentration is high. This may be due in some cases to a drop of the concentration of FSH (a natural mitogen for granulosa cells) as well as to possible prolactin inhibition of the aromatose system and, accordingly, of the intrafollicular synthesis of estradiol - another mitogenic factor for granulosa cells.

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The State of the Microcirculatory Bed, Microhemodynamics, and Oxygen Supply of the Liver under Conditions of Disrupted Parasympathetic Innervation

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Vagotomy is shown to result in disturbances of the microcirculation (a reduced rate of blood flow and distortion of its kinetics), the morphological basis of which consists of certain transformations of the microvascular network organization and ultrastructural changes in the cells lining the sinusoidal capillaries. The most pronounced disorders in microhemodynamics and blood supply of the liver are found 5-14 days after vagotomy.

Key Words: *liver; vagotomy; microcirculation; oxygen; hypoxia*

The mechanisms underlying the hypoxic state developing in organs of the digestive system in parasympathetic innervation disorders need to be specified. The data available are devoted, as a rule, to some particular aspect of the microcirculation (morphological, physiological, or biophysical [6,9, 11,14], and this hampers attempts to gain a comprehensive idea of all the regular transformations tacking place in the microhemodynamic system when the innervation is impaired and to judge the

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