

EXPERIMENTAL METHODS IN CLINICAL PRACTICE

State of Ovarian Function in Experimental Hyperthyroidism and Hyperthyrotropinemia

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The effect of experimental hyperthyroidism and hyperthyrotropinemia on histophysiological processes in the female reproductive system is studied. Thyroid hormones and thyrotropin are found to exhibit a pronounced modulatory effect on morphogenetic processes in the female gonad.

Key Words: ovary; thyroid gland; folliculogenesis

Numerous aspects of regulation of the morphogenetic basis of ovarian function have been studied at length. However, there is now a need to broaden the known basic regulating system (hypothalamus-pituitary-gonads) by adding the complex of extra- and intraorgan modulators. The specific regulators of cyclic ovarian function, the gonadotropic hormones, are complexes of hormone-regulated isoforms, the specificity of which is determined by the endocrine status of the organism. Recent investigations point to a close relationship between dysfunctions of the female reproductive and thyroid systems. The morphophysiological basis of this relationship remains to be explored. The aim of the present study was to investigate the state of the female gonad under conditions of experimentally induced hyperthyroidism and hyperthyrotropinemia.

MATERIALS AND METHODS

The ovaries of mature random-bred rats with a stable 4-day estrous cycle were studied. The animals of group 1 were repeatedly injected with triiodothyronine (T_3) in a single dose of 0.3 g/kg. In group 2 hyperthyrotropinemia was modeled by injecting

thyrotropic hormone (TSH) in single doses of 0.05 IU. The above interventions were started in some animals from proestrus and in others from the first day of diestrus. The characteristics of a single cycle were analyzed. The control animals were injected with the same volume of vehicle.

In some animals ovulation was artificially stimulated to elucidate the mechanisms of ovarian dysfunction. Changes in the cycle were judged from vaginal smears. Serial histological slices were studied using a routine histological method as well as the method of differential follicle count (according to a published classification [2]). The hormone content in the peripheral blood was determined by radioimmunoassay.

RESULTS

Out of 33 rats injected with T_3 starting from proestrus the cycle remained unchanged in 15 and permanent diestrus (anovulation) was established in 18 animals. Out of 15 rats injected with T_3 starting from diestrus the onset of permanent diestrus occurred in 10 and the physiological cycle was preserved in 5 rats. On day 4 of permanent diestrus the histological structure of the ovaries was analyzed and follicles of various stages were counted. Radioimmunoassay of the T_3 content in the blood

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samples verified hyperthyroidism in the experimental rats. In the gonads of the experimental animals in comparison with the controls (intact rats, day 2 of diestrus) we found an increased content of growing (345.7 ± 9 vs. 320 ± 9.4 , $p < 0.05$) and late preantral (155.4 ± 5.5 vs. 135.7 ± 3.5 , $p < 0.05$) follicles, while the number of medium and large antral follicles was reliably lower than in the control (18 ± 3.6 vs. 50 ± 4.6 , $p < 0.001$, and 4.2 ± 1 vs. 12 ± 2 , $p < 0.05$, respectively). These data suggest a delayed transition of growing and late preantral follicle populations to later stages of growth, which leads to an accumulation of these follicles, their partial atresia, and depletion of the pools of medium and large follicles. Some medium and large antral follicles were cystically altered and were not taken into account in the morphometric analysis. This transformation of follicles was not accompanied by luteinization of the granular and thecal layers. The pool of resting follicles was also depleted in comparison with the control. A large number of microcysts without signs of luteinization of the adjacent stroma was observed.

For a study of the possible effect of hyperthyroidism on the gonadotropin system, hormonal stimulation of ovulation was performed in some animals with prolonged diestrus. In the majority of them (8 out of 10) after a prescribed interval we observed proestrus-specific signs in the vaginal smears and active growth of ovarian follicles and preovulatory signs (vasodilation and interstitial edema) in the ovaries. In the gonads of these animals obtained on the first day of estrus we found new corpora lutea, indicating that ovulation had occurred. The results of the induction of ovulation in the experimental animals suggest that hyperthyroidism has an effect on the ovaries, while folliculogenesis is mediated primarily through the gonadotropin system, which interferes with the entry of the late preantral follicles into the stage of rapid gonadotropin-dependent growth, while medium and large follicles under conditions of gonadotropin deficit undergo cystic-atretic transformation. This results in anovulation and permanent diestrus.

When TSH was injected during proestrus, diestrus was recorded in the vaginal smears in all experimental animals 24 hours postinjection. At this time the ovaries were excised. In comparison with the control (day 1 of physiological diestrus) the pools of both medium and large antral follicles were decreased (20.4 ± 2.7 vs. 36 ± 1.3 and 5 ± 1 vs. 10 ± 1 , respectively, $p < 0.05$), despite the fact that in the control the number of large antral follicles was additionally decreased due to ovulation. The number of resting, growing, and late preantral follicles was close to the control values. In the ovaries of

the experimental animals we observed follicular cysts with signs of luteinization of the granulosa. Since the effect of TSH on folliculogenesis in proestrus was similar to that of T_3 , it may be assumed that the effect of TSH on the ovaries is mediated via positive feedback by an elevated concentration of thyroid hormones. The plasma concentration of T_3 and thyroxine in the experimental rats was identical to that in the control. It may be assumed that a single injection of TSH on the day of proestrus is not sufficient to interfere with hormone production by the thyroid gland. However, the intraperitoneally injected TSH might have exerted a direct effect on the gonad.

The fact that cystic transformation of the large preantral follicles was attended by marked luteinization of the granulosa also suggests that the mechanism of the effect of hyperthyrotropinemia on the ovary differs from that of exogenous injection of T_3 . *In vitro* experiments have shown that TSH, being similar in chemical structure to luteinizing hormone, induces luteinization of the granulosa cells [1]. It is possible that in our experiments TSH, by potentiating luteinization of the granulosa of the antral follicles, directly induced anovulation. The fact that stimulation of ovulation in the experimental animals failed also confirms the possibility of a direct effect of TSH on the terminal stages of folliculogenesis. Repeated injections of TSH starting from the first day of diestrus induced permanent diestrus in all animals. On day 4 of diestrus the population of antral follicles was depleted in comparison with the control (day 2 of physiological diestrus): from 50 ± 4.6 to 31.8 ± 5.1 , $p < 0.05$, and from 12 ± 2 to 7.3 ± 1 , $p < 0.05$, for medium and large antral follicles, respectively. The number of late preantral follicles was decreased, while the number of growing follicles did not differ from that in the control. The atretic process was markedly activated and the mean number of atretic follicles per ovary increased from 240 ± 15 to 287 ± 13.3 ($p < 0.05$). Luteinization of the stroma in the center of the gonad was pronounced.

We performed stimulation of ovulation in some of the TSH-treated animals in permanent diestrus. The previous disturbances of the cycle and of folliculogenesis persisted in 8 out of 10 animals. It may be assumed that TSH, which possesses chemical affinity to gonadotropic hormones, competitively binds to their receptors in follicles and cells of the interstitial tissue, inhibiting preovulatory changes and ovulation and inducing luteinization of granulosa cells and stromal cells.

Thus, hyperthyroidism induced by injection of T_3 modulates the course of folliculogenesis, affecting the gonadotropin-dependent stages through the

gonadotropin system. Hyperthyrotropinemia probably provokes anovulation independently by acting directly on the ovaries.

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Effect of Native Venom of Red Cobra (*Naja pallida*) on Morphological and Rheological Properties of Erythrocytes

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The effect of native venom of red (spitting) cobra (*Naja pallida*) on human erythrocytes is studied using the methods of electron microscopy and filtration through Millipore filters. Morphological analysis reveals marked transformation of discoid erythrocytes to echinocytes. More prolonged contact of the blood with the venom impairs the deformability of erythrocytes and induces hemolysis and the sludge syndrome. The results indicate a potent cytotoxic effect of native venom of red cobra.

Key Words: erythrocytes; rheology; cobra venom

The evolution of zootoxins, and in particular snake venoms, was directed at acquisition of the capacity to selectively damage the vital integrating systems of the organism: the blood, circulatory, and nervous systems. Diffusion of the venom in the organism is facilitated due to its ability to cleave the fibrinogen molecule and considerably reduce its plasma concentration. This results in hypocoagulation, inhibition of erythrocyte aggregation, decreased blood viscosity, and accelerated magistral blood flow [8]. These properties of snake venoms were used to design the drugs arvin, ancrod, and batroxobin for the treatment of peripheral arterial diseases and for the prevention of venous thromboembolism [3]. A study of the effect of snake venoms on the erythrocyte membrane is of special interest, since erythrocytes represent a universal model of the cell membrane [2]. More-

over, erythrocytes are an indispensable component of the rheological system which is responsible for optimal functioning of the micro- and macrocirculation. The most important property of erythrocytes here is their deformability, namely, the properties governing their shape, rigidity, and elasticity.

The above considerations prompted us to study the effect of some rare venoms of elapid snakes on the morphological transformation of human erythrocytes.

MATERIALS AND METHODS

Naja pallida venom (0.1 µl, 0.028 mg dry substance) was added to 5 ml heparinized blood. The samples were incubated at 37°C for 5, 15, and 20 min. Intact heparinized blood served as the control.

For scanning electron microscopy samples of peripheral blood were fixed in 4% paraformaldehyde on a phosphate buffer (pH 7.4) and treated as de-

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