FUNCTIONAL MORPHOLOGY OF THYMOCYTES DURING PHYSIOLOGICAL FLUCTUATIONS OF THE HORMONAL BALANCE

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The view that the sensitivity of cells of the lymphoid system to hormones of both protein and steroid nature has recently been consolidated [8, 14, 15]. Most workers who have demonstrated these effects, however, have used methods which make it difficult to extrapolate their results to physiological conditions: injection of doses of hormones several orders of magnitude higher than the normal level [3, 6] or blocking plans of internal secretion [4]. The action of individual hormones on lymphocytes cultured in vitro likewise differs considerably from the resultant effect of a combination of hormones acting on lymphocytes in vivo at a given moment of time [11-14].

It was therefore decided to study metabolism of cells of the lymphoid system during physiological fluctuations in hormonal balance. The thymus was chosen as the test object because of its zonal distribution of lymphocytes of the same type, differing in their degree of maturity (from committed stem cells to mature T-lymphocytes capable of an immune response) [2]. The estrous cycle, accompanied by changes in the levels of most protein and steroid hormones [1], was used as the model of physiological fluctuations in the hormonal balance.

EXPERIMENTAL METHOD

Female albino rats aged 5 months (weighing 130-150 g), with a regular 5-day estrous cycle, were used. The animals were kept under identical conditions with strictly measured daylight: 12 h light and 12 h darkness. The phases of the estrous cycle (estrus, metestrus, diestrus, proestrus) were identified from the cytological picture of vaginal films and the histological structure of the vaginal epithelium. At each phase from five to seven animals were selected. The rats were killed by decapitation between 9 and 10 a.m. The thymus was removed within 5 min after decapitation and frozen in iso-octane, cooled in liquid nitrogen. The material was kept in a Dewar flask with dry ice at −70°C. Histochemical investigations on cryostat sections 10 μ (±1 μ) thick included the following methods: detection of total nucleic acids (NA) and DNA by gallocyanin and chrome alum at pH 1.1 (RNA was determined as the difference between the NA and DNA levels); determination of activity of NADH- and NADPH-diaphorases and of acid phosphatase, using naphthol AS-BS as the substrate).

Three zones were distinguished in the thymus: subcapsular, cortical, and medullary, corresponding to the successive stages of maturation of the thymocytes [9]; in each zone 100 cells from each animal were studied.

Enzyme activity and the NA content were estimated by photographic cytophotometry using MUF-6 and MF-2 instruments. Numerical data were processed on the ES-1020 computer with calculation of the mean values and their confidence intervals at the 95% level of probability.

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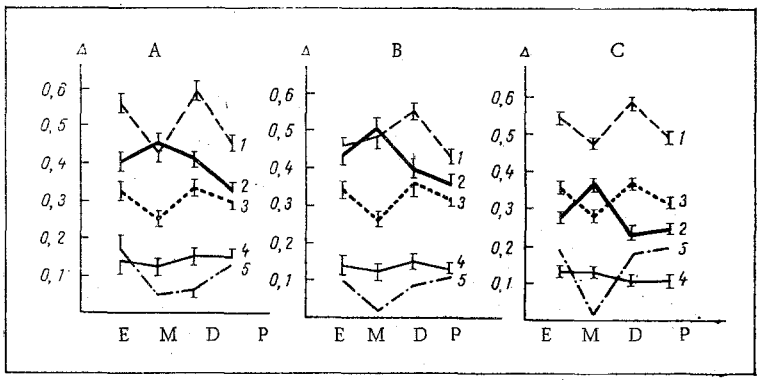

Fig. 1. Changes in activity of NADH-diaphorase (1), NADPH-diaphorase (3), and acid phosphatase (4) and in DNA (2) and RNA (5) content in thymocytes of subcapsular (A), cortical (B), and medullary (C) zones of albino rat thymus during estrous cycle. Abscissa, phases of cycle: E) estrus, M) metestrus, D) diestrus, P) proestrus; ordinate, optical density.

EXPERIMENTAL RESULTS

Analysis of the state of the principal life-support system of the thymocyte during the estrous cycle revealed both general changes, unconnected with the degree of maturity of the thymocyte, and a number of particular features characteristic of each separate zone (Fig. 1). In all zones of the lobule of the thymus reciprocal relations were observed between the DNA and RNA levels of the thymocytes, which is evidently a common feature of all actively proliferating cells [5]. Maximal fluctuations in the levels of the DNA and RNA content were observed in thymocytes of the medullary zone, probably reflecting the greater sensitivity of the information control system [1] of the mature thymocytes to changes in hormonal balance than that of immature forms. At the same time it was noted that in the diestrus – proestrus interval (phases of maximal strain on the neuroendocrine system [1]) the DNA level in the thymocytes of the subcapsular and cortical zones continued to fall, whereas in the thymocytes of the medullary zone the DNA level became stabilized. This fact may also be evidence in support of the greater sensitivity of thymocytes in the medullary zone to fluctuations in the hormone level during the estrous cycle.

The study of oxidoreduction processes in the maturing thymocytes at phases of the estrous cycle showed (Fig. 1) significantly coordinated changes in mitochondrial and extramitochondrial indices of energy metabolism. An increase in the concentrations of protein and steroid hormones in the blood serum in the phase of proestrus [1] was accompanied by a decrease in activity of oxidative enzymes in all the thymocytes regardless of the degree of their maturity. Meanwhile, as the thymocytes migrated from the subcapsular to the medullary zone, changes in activity of NADH- and NADPH- diaphorases and in the RNA content became more coordinated, evidence of the closer interlinking of the different aspects of cell metabolism in the mature cells,

An unexpected fact was that no change in acid phosphatase activity was found in the thymocytes of all the zones throughout the estrous cycle. Considering that the method of quantitative cytochemistry is nowadays a reliable, objective, and sensitive method of determination of enzyme activity [10], it can be concluded that physiological changes in hormone levels had no significant effect on the state of the vacuolar apparatus of the thymocytes, when tested by the reaction for acid phosphatase, widely used for this purpose.

It can thus be concluded from these results that the information control system and oxidoreduction enzymes in thymocytes of all zones of the thymus lobule of albino rats are sensitive to hormone fluctuations during the estrus cycle, and that thymocytes of the medullary zone are most sensitive. Meanwhile, physiological changes in hormone levels do not affect acid phosphatase activity in the thymocyte, regardless of the degree of its maturity.

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PARTHENOGENETIC DEVELOPMENT OF OVULATED MOUSE OVA UNDER THE INFLUENCE OF ETHYL ALCOHOL

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Several investigations, the results of which have been published in important surveys [1, 5, 7, 9], have been devoted to experimental parthenogenesis in mammals. Various methods of activating mouse ova for parthenogenetic development have been described [2], but a common defect of these methods is either the poor reproducibility of the results or the need to use a laborious technique for culturing oocytes in vitro.

During the study of the embryotoxic and teratogenic action of alcohol in the writers' laboratory, it was found that intraperitoneal injection of ethyl alcohol into nonpregnant mice activates the ovulating ova and induces their parthenogenetic development. These observations are described below.

EXPERIMENTAL METHOD

Experiments were carried out on 221 female hybrid (C57BL × CBA)F_1 mice, obtained at the age of 4 weeks from the "Rappolovo" nursery. Before the beginning of the experiment the animals were kept for not less than 2 weeks under conditions of 17 h daylight and 7 h darkness. The mice were mated with vasectomized males a few hours before the end of the dark period and ovulating females with vaginal plugs were selected. Under these conditions of illumination ovulation was synchronized and took place 1 h after the end of the dark period. The mice were given a single intraperitoneal injection of 0.35 ml of 25% ethyl alcohol at different times after ovulation. Alcohol (0.6 ml) was given to one group of experimental mice via gastric tube. The control group of females with vaginal plugs did not receive ethyl alcohol and was used for testing the sterility of the vasectomized males. Ova or cleaving embryos were flushed out of the genital tract of the control and experimental animals with warm medium No. 199 and investigated in the living state. Air-dried preparations

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