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QUANTITATIVE HISTOENZYMIC ANALYSIS OF THE ADENOHYPOPHYSIS AND ADRENAL CORTEX IN THE EARLY STAGES OF INVOLUTION

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To assess the changes in the neuroendocrine system during involution a method of quantitative histoenzymic analysis was used. Activity of NAD- and NADP-oxidoreductases, acid and alkaline phosphatases, glucose-6-phosphate dehydrogenase, and 3β-OH-steroid and 11β-OH-steroid dehydrogenases. Differences were found in the structural and metabolic basis for physiological adjustments of the neuroendocrine system during the course of the estrous cycle in the early stages of involution. An initial reduction in transport from cells to vessels was demonstrated in the aging animals, although functional activity of the intracellular organoids was preserved.
KEY WORDS: cytospectrophotometry; histoenzymic analysis; adenohypophysis; adrenal cortex; age involution.

Modern views on age changes in the neuroendocrine system (NES) are based principally on the results of physiological and biochemical investigations [1, 4, 7]. Only a few morphofunctional studies using quantitative cytochemical analysis have been undertaken [6, 8]. In previous investigations the writers showed that disturbances of hormonal homeostasis appear in rats at the age of 12 ± 2 months, in the form of lengthening of the estrous cycle on account of diestrus and desynchronization of the cyclic function of the ovaries, adrenal cortex, and thyroid gland [3].

In this investigation an attempt was made to analyze the morphofunctional state of the adenohypophysis and adrenal cortex in the early stages of involution in order to determine objective structural-functional criteria that could bring about the age disintegration of the NES discovered previously.

EXPERIMENTAL METHOD

Experiments were carried out on 48 female albino rats aged 12 ± 2 months (24 animals) and 4-5 months (24 animals) using 6 rats for each phase of the cycle. The activity of the following enzymes was determined in cryostat sections (10 μ thick) through the pituitary and adrenal glands: NAD- and NADP-oxidoreductases (NADO, NADPO) and acid (AcP) and alkaline (AlP)phosphatases; in addition, activity of glucose-6-phosphate dehydrogenase (G6PD) and 3β-OH-steroid and 11β-OH-steroid dehydrogenases (3β-OH-SD, 11β-OH-SD) was determined in the adrenal. The intensity of the histoenzymic reactions was assessed quantitatively with the MUF-5 microspectrophotometer by scanning in visible monochromatic light. The numerical results were analyzed by special program on the EC-1020 computer [5]. Besides estimation of the mean value and dispersion, mathematical analysis of the results of cytospectrophotometry included comparison of histograms, giving the minimal percentage of cells in which enzyme activity changed during the transition from one phase of the estrous cycle to another [2].

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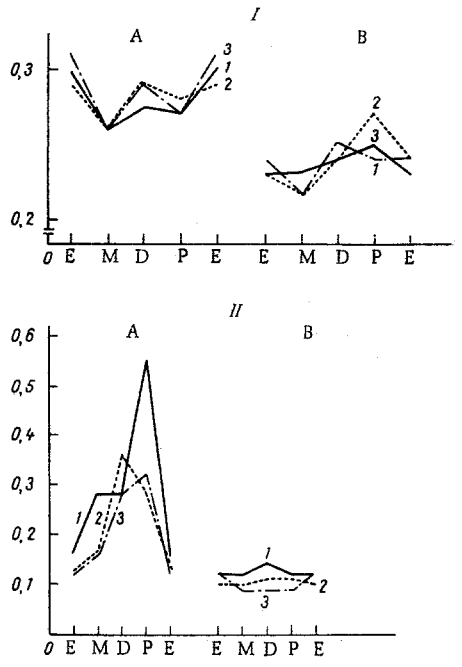

Fig. 1

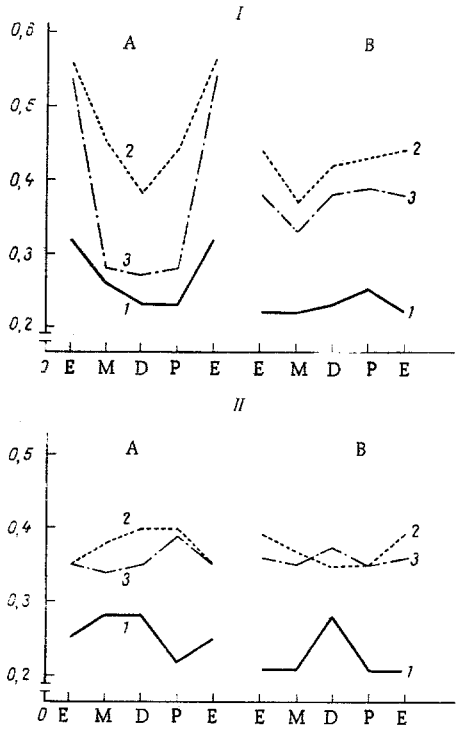

Fig. 2

Fig. 1. Dynamics of enzyme activity in adenohypophysis of rats of two age groups during estrous cycle. I) NADPO; II) AlP; A) young animals; B) aging animals; 1) ventral, 2) central, 3) dorsal part of adenohypophysis. Abscissa, phases of estrous cycle: E) estrus, M) metestrus, D) diestrus, P) proestrus; ordinate, enzyme activity (in optical density units).

Fig. 2. Dynamics of enzyme activity in adrenal cortex of rats of two age groups during estrous cycle. I) G6PD; II) 3β-OH-SD; 1) zona glomerulosa (outer); 2) zona fasciculata (middle); 3) zona reticularis (inner). Remainder of legend as in Fig. 1.

EXPERIMENTAL RESULTS

Enzyme activity changed in all parts of the adenohypophysis of the young animals. Increased activity of the enzymes of energy metabolism (NADO, NADPO) was observed in 20-30% of cells in all parts of the adenohypophysis. In the ventral and dorsal parts, changes in enzyme activity were in the same direction. In the cells of the central part changes in cell function were in different directions: Both an increase and a decrease in enzyme activity were observed. In the central and dorsal parts, changes in enzyme activity were synchronized in the mitochondria and hyaloplasm, whereas in the ventral part activation of mitochondrial enzymes (the NAD-dependent group) in the phase of proestrus was accompanied by a decrease in enzyme activity in the hyaloplasm (the NAD-dependent group). In all zones of the adenohypophysis the cyclic character of the change in activity of the enzymes of energy metabolism was similar both in cells in contact with blood vessels and in intratrabecular cells. In all parts of the adenohypophysis high AcP and AlP activity was observed in diestrus–proestrus (Fig. 1, IIA), evidence of intensification of transport processes in the vessel wall (AlP) and of lysosomal function of the adenohypophyseal cells (AcP).

In the adrenal cortex of the young rats the dynamics of activity of the enzymes of energy metabolism (NADO, NADPO, G6PD) was as follows: In the zona glomerulosa enzyme activity was changed in 40% of cells, in the zona fasciculata in 50%, and in the zona reticularis in 80%. The characteristic feature of all energy enzymes was a sharp change in activity in only one phase and maintenance of a relatively stable level during the rest (Fig. 2, IA). The reason for this character of the change in enzyme activity in all the zones was a unidirectional change in the functional state of the cells, whereas in the period of relative stabilization both an increase and a decrease in enzyme activity were observed in the cells. Zonal differences also were clearly defined in the cyclic fluctuations of activity of the enzymes of specific steroid synthesis (3β-OH-SD, 11β-OH-SD).

A change in 3β-OH-SD activity in the zona glomerulosa was due to an increase in the functional state of 40-50% of the cells, whereas in the zona fasciculata and zona reticularis the increase occurred in 20-30% of cells. In the course of the cycle, 3β-OH-SD activity rose gradually during three phases (Fig. 2, IIA), and the accompanying changes in the state of the cells could be in either the same or different directions. Cyclic changes in 11β-OH-SD activity were brought about in 50-60% of cells in the outer parts of the cortex, and in 30-40% of cells in the inner part of the zona fasciculata and in the zona reticularis. In the outer parts of the cortex changes in the functional state of the cells were always in the same direction, whereas in the inner parts 11β-OH-SD activity in the cells could either increase or decrease.

In the adenohypophysis of the aging animals a very small decrease in the mean activity of all enzymes studied was observed despite preservation of cyclic changes in the functional state of the cells (Fig. 1, IB). However, the direction of the cyclic dynamics varied: Activity of the enzymes of energy metabolism was depressed at the beginning of the cycle, whereas in young rats it was activated. A new mechanism of structural support for the cyclic dynamics of the tropic functions of the pituitary was revealed. This was expressed primarily as a disturbance of integration of the functions of the different parts: a more marked decrease in all criteria of activity in the adrenocorticotropic zone than in the thyrotropic and gonadotropic zones. For instance, a cyclic change in the activity of the enzymes of energy metabolism in the ventral and central parts was brought about by an increase in the functional state of 40-50% of the cells, compared with only 20-30% in the dorsal part. In all parts of the pituitary, but more especially the dorsal, the cyclic character of the change in AlP activity became less conspicuous and the fluctuations in its activity amounted to only one fifth of those in the young rats (Fig. 1, IIB).

In the adrenal cortex of the aging rats the mean activity of all enzymes tested was the same as in the young animals. Age differences were expressed primarily as the almost complete disappearance of the cyclic dynamics of activity of the enzymes of energy metabolism (Fig. 2, IB), maintained by only 20-40% of the cells. No distinct zonal differences such as were characteristic of the young animals could be detected, but a relative increase in enzyme activity was observed in the zona reticularis. The magnitude of the cyclic changes in the activity of the enzymes of specific steroid synthesis was similar to that in the young animals, but in the old animals the direction and character of the interzonal relationship were altered. Zonal differences in 3β-OH-SD activity tended to disappear: The cyclic dynamics was maintained by 20-40% of cells in the whole cortex. With respect to 11β-OH-SD activity the function of the zones was completely reversed: The maximal dynamics of the enzyme (50-60% of cells) was observed in the inner parts of the cortex and minimal (30% of cells) in the outer parts.

During quantitative cytochemical analysis of the pituitary and adrenal cortex in the animals of two age groups changes were thus found in the structural and metabolic basis for the mechanism of physiological adjustment of the NES (the estrous cycle) in the early stages of involution. Despite preservation of the initial magnitude of the basic metabolic indices in both glands, the cyclic dynamics were caused by a different cell and tissue substrate. The number of cells maintaining physiological fluctuations in the processes of hormone synthesis was changed and the relative integration of the intraglandular structures was disturbed. For instance, in the adenohypophysis there was a relative decrease in the volume of the tissue structural changes in the adrenocorticotropic zone compared with the thyro- and gonadotropic zones, whereas in the adrenal the function of the inner parts of the cortex was activated. In the pituitary, a sharp decrease in the cyclic dynamics of AlP activity was discovered, and was most marked in the adrenocorticotropic zone. Meanwhile preservation of high activity of the enzymes investigated in both endocrine glands of the aging animals is evidence of the absence of any intracellular functional or morphological changes. Depression of the cyclic dynamics of activity of the enzymes responsible for the initial stages of steroid synthesis (G6PD, NADPO) points to depression of the central stimulation of steroid synthesis.

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