QUANTITATIVE HISTOENZYMOLOGIC CHARACTERISTICS OF THE SUBMAXILLARY SALIVARY GLANDS DURING THE OVARIAN CYCLE IN ALBINO RATS

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To study the connection between the salivary and endocrine glands and also the degree of participation of individual components of the salivary glands with different functions in endocrine regulation, the ovarian cycle was used. The concentrations of proteins, mucopolysaccharides, DNA, and RNA, and activity of NAD- and NADP-diaphorases, alkaline phosphatase, malate and isocitrate dehydrogenases, and α-leucine-aminopeptidase in the submaxillary salivary glands were investigated. It was shown by cytospectrophotometric analysis that, depending on the phases of the ovarian cycle, synchronous changes in the activity of the enzymes studied took place in all parts of the salivary glands. Of the four consecutive phases of the sex cycle the greatest activity of the enzymes and concentration of proteins and mucopolysaccharides were observed in proestrus and metestrus. The metabolic processes in the salivary tubules were rather different from those in other parts of the glands, and this may be attributable to differences in the pattern of secretion production and also, possibly, hormone formation.

KEY WORDS: salivary gland; hormones; enzymes; ovarian cycle.

The notion that the salivary glands have internal secretory activity and are connected with other endocrine glands is based on many clinical observations [4, 6]. Evidence has been obtained that the salivary glands are closely connected with the endocrine glands, including the gonads, as shown by the presence of sexual dimorphism of the salivary glands, and their changes after castration and in various hypogonadal states [7, 9]. Investigation of the character of changes in the salivary glands during functional disturbances of various endocrine organs has shown that the greatest changes take place in the salivary tubules [5, 10] where, according to some workers, the hormones parotin and insulin [6, 8], which participate in endocrine regulation, are produced.

To study the connection between the salivary and endocrine glands and also the degree of participation of individual components of the salivary glands with different functions in endocrine regulation, an experimental model based on the principle of minimal intervention was used, namely the ovarian cycle, during the normal course of which cyclic fluctuations are observed in the blood hormone levels due to functional changes in all the endocrine glands [2].

EXPERIMENTAL METHOD

Experiments were carried out on 24 Wistar rats weighing 130-150 g kept under standard conditions of illumination and with a regular 4-day ovarian cycle, as shown by examination of vaginal smears. The necessary precautions were taken when collecting, keeping, and investigating the material [1]. To estimate the functional state of the salivary glands during physiological fluctuations in the hormone concentrations a series of histochemical tests was used: activity of alkaline phosphatase (AP), esterase, α-leucine-aminopeptidase (α-LAP), NAD- and NADP-diaphorases, and malate and isocitrate dehydrogenases (MD and ICD) was determined, the PAS reaction performed by Hotchkiss' method to detect mucopolysaccharides, the Danielli–Pearse–Burstone reaction was carried out to detect simple proteins, and the DNA and RNA concentration was determined by staining with gallocyanin and chrome alum in frozen sections to a thickness of 10μ. The cytospectrophotometric estimation of enzyme activity and concentration of proteins and mucopolysaccharides in the various struc-

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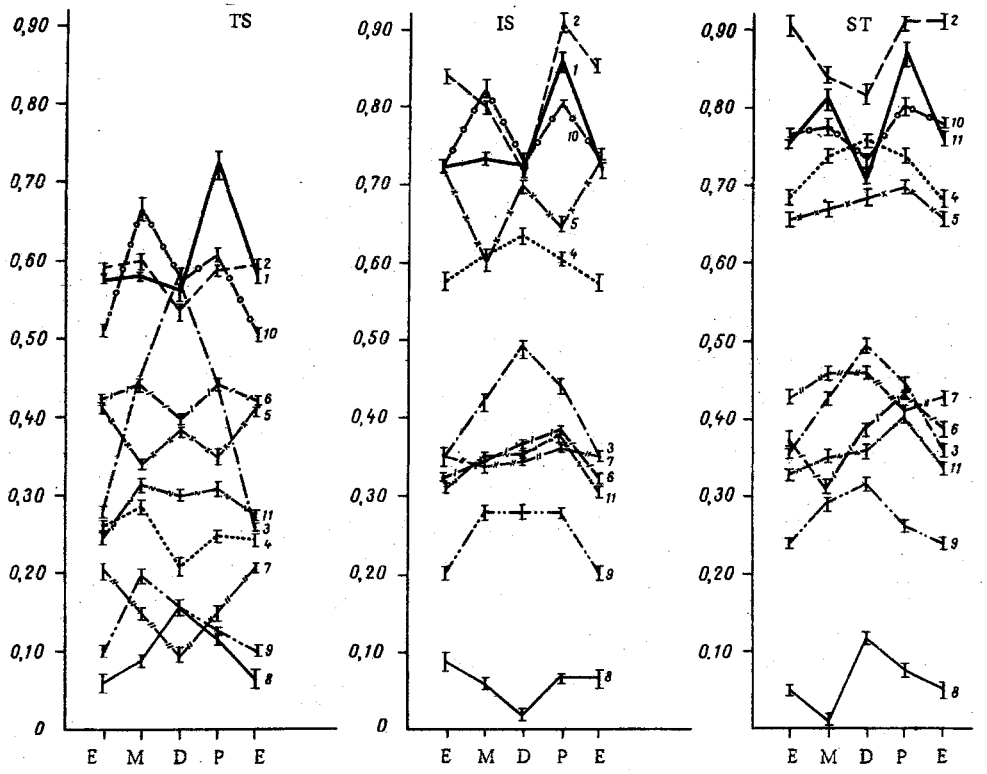

Fig. 1. Changes in enzyme activity and mucoprotein content in submaxillary salivary glands of rats during the ovarian cycle. TS) terminal segments; IS) intercalated segments; ST) salivary tubules. 1) PAS-reaction; 2) ICD; 3) AP; 4) NADP; 5) esterase; 6) DNA; 7) NAD; 8) RNA; 9) MD; 10) proteins; 11) α-LAP. Abscissa, phases of estrous cycle: E) estrus, M) metestrus, D) diestrus, P) proestrus; ordinate, optical density of reaction products (in optical units).

tures of the salivary glands (Fig. 1) was carried out by photometry of negatives obtained on the MUF-6 apparatus, after which the numerical results were subjected to computer analysis in accordance with a special program [3]. Indices of mathematical expectancy and dispersion and the standard deviation of these estimates were calculated. The significance of differences between the means was judged within a 95% confidence interval. Histograms of distribution of probabilities were analyzed and the degree of their difference determined with respect to a conventional index reflecting the minimal percentage of cells whose activity changed during transitions between the successive phases of the cycle (estrus, metestrus, diestrus, and proestrus).

EXPERIMENTAL RESULTS

Comparative analysis of the intensity of the histoenzymic reactions revealed cyclic changes in the activity of the enzymes studied in all parts of the salivary glands depending on the phases of the ovarian cycle and, correspondingly, on the blood sex steroid levels. In the terminal secretory regions, producing mainly an exocrine secretion, in the phase of proestrus when secretion of estrogens reaches a maximum an increase was observed in the mucopolysaccharide concentration, accompanied by a smaller increase in the protein concentration and by activation of enzymes serving as nonspecific indicators of synthetic processes (NADP-diaphorase and ICD) and energy metabolism (NADP-diaphorase) in the cell and by changes in the functional state of the DNA-RNA system. Under these circumstances a reduction in esterase and AP, which are hydrolytic enzymes and indicators of the functional state of the lysosomes, was observed in this phase. The rise in progesterone secretion at the end of estrus and in metestrus evoked an increase in the protein concentration and a very small increase in the mucopolysaccharide concentration, as well as activation of NADP-diaphorase, MD, α-LAP, ICD, and AP in the terminal secretory portions of the gland. The decrease in estrogen secretion during estrus was reflected in a decrease in the protein and mucopolysaccharide concentrations in these regions and a reduction in the activity of the hydrolytic enzymes of the cell. The activity of the enzymes of energy production and of synthesis showed only very small changes compared with proestrus, whereas NAD-diaphorase activity was significantly increased. In the phase of diestrus the protein and mucopolysaccharide concentrations were reduced and this was accompanied by a synchronous decrease in the activity of nearly all enzymes studied, except AP and esterase.

In the intercalated ducts, which besides reabsorbing NA carry out protein synthesis, just as in the terminal secretory portions, changes in the activity of the enzymes studied and in the concentrations of proteins and mucopolysaccharides – the principal components of the secretion produced – were observed depending on the phases of the sex cycle. The increase in the protein and mucopolysaccharide concentration and the higher esterase and NADP-diaphorase activity than in the terminal portions in all phases of the cycle will be noted.

In the salivary tubules an increase in the mucopolysaccharide concentration also was observed in the phases of metestrus and proestrus. Fluctuations in the protein concentration depending on the phases of the ovarian cycle were not significant. A characteristic feature of the salivary tubules was that the increase in the mucopolysaccharide and protein concentrations was accompanied by a decrease of NAD- and NADP-diaphorase and MD activity and by an increase in the activity of hydrolytic enzymes responsible for intracellular transport and the final formation of the protein secretion – granule formation and anabolic processes. The study of the relations between DNA and RNA in the salivary tubules of the submaxillary salivary glands revealed synchronous fluctuations in the activities of DNA and cytoplasmic RNA.

Besides the constant basal secretion, during the cycle a phase of accumulation and a phase of active discharge of secretion could be observed in all regions of the gland. Analysis of the numerical data for the optical density of the histoenzymological reaction products, which reflect activity of the enzymes, showed the heterogeneity of the structures investigated. In different cells in the same part of the salivary gland, variation was observed in enzyme activity. Mathematical analysis (plotting distribution curves) showed that in each part there was a group of cells in which the intensity of the reactions could exceed the mean level by nearly 50-100%. It can be concluded from this mosaic pattern of enzyme activation that stimulation of secretory activity of the salivary glands and at definite phases of the ovarian cycle takes place through activation not of the whole parenchyma of the organ, but only of individual cell groups.

Investigation of the group of enzymes responsible for both the initial processes of enzyme synthesis and its final stages revealed fluctuations in their activity during the ovarian cycle. Of all the consecutive phases of the sex cycle, the highest enzyme activity and the highest concentration of proteins and mucopolysaccharides in the terminal and intercalated portions were observed during proestrus and metestrus, whereas in the salivary tubules a significant increase was observed only during the phase of proestrus.

Analysis of the results indicates that functional changes in the salivary glands during the ovarian cycle are accompanied by synchronous changes in energy metabolism and synthetic processes in the cells of the terminal and intercalated portions of the ducts and by somewhat different metabolic changes in the salivary tubules.

Higher activity of the enzymes of energy metabolism in the cells and also of enzymes participating in the formation of secretory granules was observed in the salivary tubules than in other parts of the gland. This distinguishes the salivary tubules from the other parts and it is evidently connected with the special characteristics of secretion formation and also, possibly, of hormone formation. However, the site of synthesis and secretion of parotin cannot be deduced from the results. Synchronous fluctuations in enzyme activity and protein and mucopolysaccharide concentration in the terminal and intercalated ducts and the salivary tubules depending on the physiological blood hormone levels are evidence in support of the view expressed by Takana Kunio et al., that the precursor of parotin is formed in the acinar cells and is then reabsorbed by the cells of the ducts (the intercalated segments and the salivary tubules), and is transformed by them into parotin.

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