FUNCTIONAL MORPHOLOGY OF THE SUBMANDIBULAR SALIVARY GLANDS OF RATS WITH AGE DISTURBANCES OF ENDOCRINE CONTROL

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An important problem in modern medicine and biology is the study of the mechanisms of aging and the possibility of development of diseases connected with them. There is much evidence of age changes in structural and histochemical parameters of salivary gland function in man and different species of animals [6, 16, 20]. At the same time we know that certain diseases of the salivary glands which, as we know, perform an endocrine function [1, 7], and in particular, metabolic sialoses and autoimmune paratitis may arise when the integrity of the endocrine system is disturbed, most frequently in the period of age involution [9, 13, 17].

The aim of the present investigation was to study structural and metabolic parameters of salivary gland function in old rats with different degrees of disturbance of endocrine homeostasis.

EXPERIMENTAL METHOD

Experiments were carried out on 160 female albino rats aged 20.5 months, in which either the reproductive cycle had ceased with the onset of permanent estrus or diestrus, or the length of the cycle was sharply increased. Phases of the estrous cycle were determined on the basis of the cytological picture of vaginal smears and the histological structure of the vaginal epithelium.

Dyshormonal hyperplasias of the mammary gland which, as we know, result from the disturbance of steroid homeostasis at tissue and organ levels [2, 12], served as the morphological criterion of the depth of disturbance of the hormonal balance. The frequency of development of tumors and pretumor hyperplasias in the mammary glands depended significantly on disturbances of the estrous cycle. On the basis of changes in the mammary glands the following groups of rats with different degrees of disturbance of the hormonal balance were distinguished [3]: group 1) normal age involution of the mammary gland, 2) initial form of dyshormonal hyperplasia of the parenchyma and stroma, 3) the proliferative form of dyshormonal hyperplasia, 4) dyshormonal tumors of the mammary glands.

To assess the functional state of the salivary glands a combination of histochemical methods was used. All the necessary requirements were met when the material was taken, kept, and investigated [4]. Activity of alkaline and acid phosphatases (AlP and AcP respectively), esterase, and NAD- and NADP-diaphorases was determined, the PAS reaction performed, and the concentrations of DNA and RNA determined by staining with gallocyanin in cryostat sections 10 μ thick. Activity of the enzymes and the protein concentration were determined cytospectrophotometrically in 50 cells in the terminal secretory portions, the intercalated ducts, and the salivary tubules, which perform different functions; the analysis was made by photometry of negatives obtained on the MUF-6 instrument, followed by computer processing of the material data [5]. The statistical distribution hypotheses were evaluated by the Mann–Whitney nonparametric test and the closeness of the relationship between the nucleic acid concentrations in the nucleus and cytoplasm to a linear function was assessed by the coefficient of correlation.

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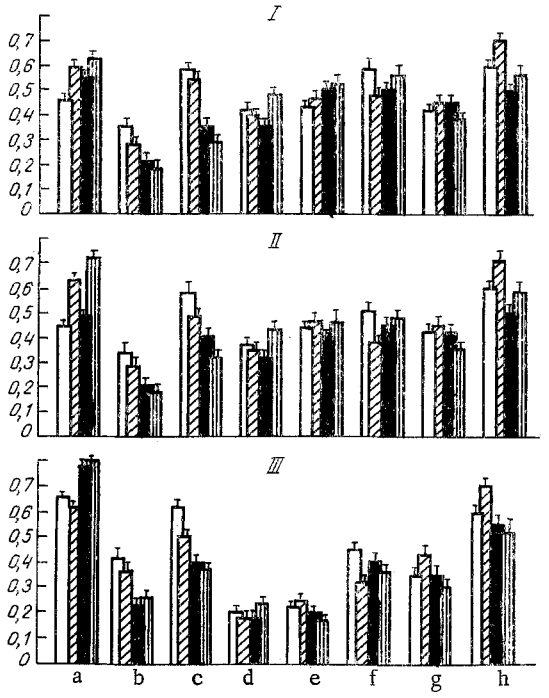

Fig. 1. Changes in enzyme activity and protein concentration in salivary glands of rats with different degrees of disturbance of endocrine control. Abscissa, enzyme activity (in relative optical density units). Unshaded columns — animals of group 1, obliquely shaded columns — group 2, black columns — group 3, vertically shaded columns — group 4. I) Salivary tubules, II) intercalated ducts, III) terminal secretory portions. a) PAS reaction, b) RNA, c) DNA, d) NADP, e) NAD, f) esterase, g) AcP, h) AlP.

EXPERIMENTAL RESULTS

In old rats with different disturbances of the estrous cycle visual examination of the submandibular salivary glands revealed no particular features in the character of distribution and activity of the various test substances. The results of the photometric investigation revealed some general rules and demonstrated a definite time course in the character of metabolism (Fig. 1).

Terminal Secretory Portions. During the study of the terminal secretory portions the content of mucoproteins, a basic parameter of secretory function, was higher in the salivary glands of rats of groups 3 and 4, characterized by the severest disturbances of their endocrine system. If this increase in the mucoprotein content in the animals of the above groups is regarded as potentiation of their secretory function, it must be maintained by an increased supply of energy and an increased content of nucleic acids, especially cytoplasmic RNA, as is observed in young animals [8]. However, analysis of nucleic acids in the salivary glands of rats of groups 3 and 4 showed that their content of DNA and cytoplasmic RNA was significantly lower than in the animals of groups 1 and 2. The coefficient of correlation indicates a direct relationship between the nucleic acid content in the nucleus and cytoplasm only in the rats of group 1. In the animals of the other three groups, only in some cases could correlation be observed between these parameters, i.e., in most cases there was nucleo-cytoplasmic dissociation. The study of the NADP-diaphorase content, a parameter of the energy supply of the endoplasmic reticulum, revealed very slight changes with time, with a small fall in the level of the enzyme in rats of group 4. The nonparametric Mann–Whitney test showed that this decrease in NADP-diaphorase activity is not accidental. Activity of NAD-diaphorase, a marker of mitochondrial energy metabolism, was characterized by a significant fall in the animals of groups 3 and 4 compared with the corresponding parameter in the rats of group 1. During investigation of the state of the wall of the microcirculatory system, reflected in AlP activity, an increase in the content of the enzyme was observed in the animals of group 2 and a decrease in the rats of groups 3 and 4.

During investigation of AcP and esterase activity in the terminal secretory portions, the highest esterase content was found in the animals of group 1. AcP activity was maximal in the rats of group 2; rats of groups 3 and 4 were characterized by lower activity of their lysosomal enzymes than those of group 1. The study of the intralobular duct system showed the following pattern. A higher content of mucoproteins also was observed in the rats of groups 3 and 4 compared with the animals of group 1. A lower level of DNA and RNA was found in rats of groups 3 and 4. The closest correlations were found only in animals of group 1, and in all other groups dissociation of nucleo-cytoplasmic ratios was observed. NAD- and NADP-diaphorase activity in the duct system of the salivary glands of the rats of groups 3 and 4 was characterized by little change with time, during accumulation of secretory products in the cells. AlP activity behaved in the same way as in the terminal portions: it was depressed in the rats of groups 3 and 4 and increased in those of group 2. Esterase and AcP activity was low in animals of all groups except group 1. Statistical analysis showed that the order of the changes thus revealed in the duct system is not accidental. Consequently, changes similar to those in the terminal secretory portions, but less marked, arise in the intralobular duct system of the salivary glands during age disturbances in the endocrine system.

The character of metabolic processes thus changes with age in different parts of the salivary glands of albino rats. Age disturbances of the endocrine system, manifested at the tissue level by changes in hormone-dependent organs, lead to the development of new interrelationships in the salivary glands. These may lead to dissociation between the processes responsible for synthesis of the principal secretory products and their elimination from the cell. Different degrees of disorganization in the endocrine system are reflected also in the character of changes in the salivary glands. A very small reduction in the volume of synthetic processes was observed in the salivary glands of the rats of group 1 and relations between the parameters reflecting this process remained adequate. In animals with initial signs of disturbance of endocrine regulation (group 2) the changes were more marked, but high activity of the lysosomal enzymes led to increased degradation of secretory granules (the phenomenon of "crinophagy" [11, 14]), which accumulated in the cell as a result of disturbance of transport processes. When more profound structural changes were present in the endocrine system (groups 3 and 4), intracellular transport and catabolism were disturbed in the salivary glands (reduction in AcP and esterase activity), nucleo-cytoplasmic dissociation developed, activity of the enzymes responsible for vascular transport was depressed, with the result that secretory stasis arose. Consequently, disturbance of the process of liberation of secretion from the cell when activity of lysosomal enzymes was depressed (the possibility of injury to the lysosomes has been demonstrated during changes in steroid homeostasis [10]) may be the trigger mechanism for onset of a state of "delay in the phase of storage of secretory granules" [19], observed during the development of metabolic sialosis. The fact that pathological changes develop in the salivary glands and increase in severity during progression of dyshormonal hyperplastic processes in the target organs is confirmation of the hypothesis that the salivary glands, which also perform an independent endocrine function of their own in rats, are themselves hormone-dependent [16, 18].

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TIME COURSE OF CONTENT OF CATIONS AND ORGANOPHOSPHORUS COMPOUNDS IN ERYTHROCYTES AFTER LOW-TEMPERATURE PRESERVATION (—196°C) WITH 1,2-PROPANEDIOL AND GLYCEROL

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For long-term low-temperature preservation of blood cells in the USSR [1, 2] and elsewhere [8, 10] protective media containing glycerol are most frequently used. However, the need to remove the glycerol from the cells before they are transfused places limitations on the use of this method, and for that reason the search is in progress for less laborious and more effective methods of preservation. In this respect a method of low-temperature preservation of erythrocytes under protection of 1, 2-propanediol (1,2-PD), developed at the Institute for Problems in Cryobiology and Cryomedicine, Academy of Sciences of the Ukrainian SSR, which essentially simplifies the procedure of removal of the cryoprotector from the preserved cells before use, is interesting.

This paper gives the results of a comparative study of the time course of the concentrations of ATP, 2,3-disphosphoglycerate (2,3-DPG), and also of Na^+ and K^+ ions in erythrocytes after preservation for five days at 4°C, in suspending media 8b and 8c (Central Research Institute of Hematology and Blood Transfusion) after low-temperature preservation with 1,2-PD are described in this paper. It can be postulated that this protection is due to the fact that the 1,2-PD remaining after washing takes part in cell metabolism, for we know that this compound, which is converted into monohydroxyacetophosphate, can take part in the glycolytic cycle [7] and act later as a source of ATP. The greater fall in the ATP level during the first 36 h of storage of depreserved cells frozen with glycerol, compared with cells frozen with 1,2-PD, indicates that the residual amounts of these penetrating cryoprotectors are involved in erythrocyte metabolism by different mechanisms, and to some extent they confirm data [9] showing that the inhibitory action of glycerol on the ATP level is reversible.

Investigation of the time course of the 2,3-DPG content (Fig. 1D), just as in previous experiments, revealed higher values in control samples suspended in media 8c. This became particularly noticeable with an increase in the length of keeping. In experimental samples suspended after heating both in medium 8b and in medium 8c, the 2,3-DPG content also was lower after all periods of keeping than in control samples suspended in medium 8c. This difference was smaller at the earlier times of keeping.

Judging from the time course of the biochemical parameters, blood preserved with 1,2-PD is comparable in quality with that preserved with glycerol, and in the early periods of

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