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MORPHOMETRIC FEATURES OF THE RESTRUCTURING OF RAT SUBMANDIBULAR ACINI UNDER THE INFLUENCE OF A COMPLEX OF FOOD ADDITIVES

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The use of a complex of food additives leads to a change in the structural and functional state of the acini of the submandibular salivary glands of rats, which was expressed by a significant decrease in the morphometric parameters of the outer diameter, inner diameter and height of epithelial cells due to the primary endogenous action of the components of the complex of food additives with an increase in the volume of the interstitium in the early stages of the experiment, but later, the action of compensatory and restorative reactions of the body did not lead to complete recovery, as there was a constant restructuring of the secretory apparatus of seromucous cells, which was confirmed by a significant wave-like increase in the diameter of the outer and height of epithelial cells, against the background of a decrease in the diameter of the lumen. The activation of secretion is associated with the reaction of the submandibular salivary glands to a change in the nature of nutrition associated with the presence of food additives and the restructuring of the base of the serotonergic apparatus in favor of proteins, with the appearance of some areas of complete sloughing, and is a morphological confirmation of the predominance of the carbohydrate component, and is protective in relation to the effect of the components of the food additives complex on the mucous membrane of both the oral cavity, and the initial parts of the gastrointestinal tract.

Key words: food additives, monosodium glutamate, sodium nitrite, Ponceau-4R, salivary glands, terminal parts, rats.

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МОРФОМЕТРИЧНІ ОСОБЛИВОСТІ ПЕРЕБУДОВИ КІНЦЕВИХ ВІДДІЛІВ ПІДНИЖНЬОЩЕЛЕПНИХ СЛИННИХ ЗАЛОЗ ЩУРІВ ПІД ВПЛИВОМ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК

Вживання комплексу харчових добавок глутамату натрію, нітриту натрію та Понсо- 4R призводить до змін морфометричних показників кінцевих відділів піднижньощелепних слинних залоз щурів, і на ранніх стадіях експерименту виражалось достовірним зменшенням діаметру зовнішнього, діаметру внутрішнього та висоти епітеліоцитів. В подальшому протягом експерименту спостерігалась хвилюватий характер змін морфометричних показників кінцевих відділів піднижньощелепних слинних залоз щурів, що в очевидь пов'язано з дією компенсаторно-відновлювальних реакцій організму, але повного відновлення не відбувалось, і на 8-му тижні спостерігалось достовірне збільшення всіх морфометричних параметрів, щодо показників у контрольній групі. В очевидь, складові комплексу харчових добавок, призвели до змін секреторної активності серомукозних гландулоцитів протягом експерименту та постійної перебудови секреторного апарату, так як підвищення секреторної активності пов'язане із збільшенням об'єму трансмурального транспорту рідини із судин мікроциркуляторного русла в інтерстицій, таким чином збільшення морфометричних показників – є морфологічним підтвердженням підвищення функціональної активності серомукозних клітин кінцевих відділів слинних залоз. Так як, на кінець експерименту морфометричні показники діаметру зовнішнього та висоти епітеліоцитів залишались достовірно більші, за такі у контрольній групі, це призвело до зменшення діаметру внутрішнього та виражалось процесом постійної стимуляції серомукозних клітин, що гістологічно підтверджувалось зміною базofilії на користь білкового компоненту основної частини гландулоцитів кінцевих відділів, але були виявлені окремі ділянки, де спостерігалось явище ослизнення та перебудови секреторного апарату на користь вуглеводів.

Ключові слова: харчові добавки, глутамат натрію, нітрит натрію, Понсо- 4R, слинні залози, кінцеві відділи, щури.

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The oral cavity is moistened by saliva, a complex biological fluid produced by the salivary glands. Saliva is categorized into two types: resting saliva and stimulated saliva. The oral cavity receives secretions from three pairs of major salivary glands, each contributing differently: the parotid glands produce serous saliva, the submandibular glands secrete a mixture of serous and mucous saliva from seromucous cells, and the sublingual glands secrete viscous saliva. Consequently, the saliva in the oral cavity is a mixture under these conditions [1].

The submandibular salivary glands in rats exhibit a typical structure comprising acini and excretory ducts, forming a system of tubes involved in the final stages of saliva modification [2].

The acini vary in shape, typically round, composed of pyramidal cells that can alter their secretion type based on different conditions. They are encased by a basement membrane, which forms an intricate framework crucial for maintaining the gland's architecture. Within the acini lies a lumen that connects to the network of

excretory ducts. Myoepithelial cells typically reside between the basement membrane and the plasma membrane [1]. Thus, the study of the influence of various factors on the structure of the salivary glands is of great importance, since the state of the oral cavity directly depends on them [14], which is known to reflect not only the dental condition but also the state of health of the body in general.

In light of current concerns, the use of food additives has become increasingly critical. Manufacturers often employ these additives extensively to mask inferior or excessively priced raw materials, occasionally disregarding the standards set forth by the State Standard of Ukraine.

Our investigation into the presence of food additives in both domestic and international products identified monosodium glutamate, sodium nitrite, and Ponceau-4R as the most prevalent. Therefore, studying the impact of these substances on the morphological and functional integrity of various organs and systems holds significant practical relevance. According to the literature, the effect of many food additives has been established [3, 5, 6, 7, 8, 10, 12], but there are no data on their combined effect, and manufacturers often use them in combination.

The purpose of the study was to determine the dynamics of changes in morphometric parameters of the acini of the submandibular salivary glands of rats in normal conditions and under the complex effect of food additives – sodium glutamate, sodium nitrite and Ponceau-4R.

Materials and methods. In the study, 84 sexually mature male rats were used. Animals of the control group consumed drinking water and received oral saline solution. The rats of the experimental group, under conditions of free access to water, were administered 0.6 mg/kg of sodium nitrite, sodium glutamate at a dose of 20 mg/kg, and 5 mg/kg of Ponceau 4R in 0.5 ml of distilled water once a day orally, with the doses of food additives being half the maximum allowable rate. The adaptive behavior of rats was assessed using the open field test. Animals were withdrawn from the experiment after 1, 4, 8, 12 and 16 weeks by an overdose of thiopental anesthesia. After euthanizing the animals, fragments of the submandibular salivary glands were fixed in a 10 % formalin solution. Then, the pieces of the mandibular salivary glands were embedded in paraffin according to the generally accepted method [13]. Sections 5-10 μm thick were obtained using an ARM 3600 microtome. After staining with hematoxylin and eosin, the sections were placed in polystyrene and examined in a light microscope. Using a digital microscope with a Levenhuk D740T digital microphotomount, microphotography and morphometric studies were performed using programs adapted for these studies. Statistical processing of morphometric data was performed using the Excel program [4, 9].

All animal experiments were carried out in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (Strasbourg, 1986), in accordance with the rules for keeping experimental animals established by the European Parliament and Council Directive (2010/63/EU) and the Order №134 of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012, No. 249 "On approval of the procedure for conducting tests, experiments on animals by research institutions", as well as the recommendations of the First National Congress of Ukraine on Bioethics (2001).

Results of the study and their discussion. Morphometric study of the acini of the submandibular salivary glands of rats showed that the average outer diameter was $33.23 \pm 0.25 \mu\text{m}$, the average inner diameter was $4.58 \pm 0.09 \mu\text{m}$, and the average height of epithelial cells was $14.11 \pm 0.09 \mu\text{m}$ (Table 1).

Table 1

Morphometric parameters of the submandibular acini of rats

Parameters.	Outer diameter (microns)	Inner diameter (microns)	Epithelial cell height (μm)
Control	33.23 ± 0.25	4.58 ± 0.09	14.11 ± 0.09
1 week	22.04 ± 0.20 *	2.76 ± 0.08 *	9.02 ± 0.14 *
4 weeks	27.61 ± 0.18 * **	2.64 ± 0.07 *	11.96 ± 0.11 * **
8 weeks	40.27 ± 0.47 * **	4.05 ± 0.06 * **	17.65 ± 0.08 * **
12 weeks	37.60 ± 0.53 * **	1.54 ± 0.02 * **	14.48 ± 0.09 * **
16 weeks	40.73 ± 0.79 * **	2.92 ± 0.03 * **	14.65 ± 0.14 *

Notes: * – $p < 0.05$ compared to the control group; ** – $p < 0.05$ compared to the previous observation period.

Histological examination of the submandibular salivary glands of the control group rats showed a lobular structure, the alveolar acini were separated by thin layers of interstitial spoliation tissue with a well-

developed network of microcirculatory vessels. The acini were formed by seromucous exocrinocytes, which had a pyramidal shape, and nuclei located in the basal part of the cells. In the apical part of the cells there were secretory granules, optically lighter than the cytoplasm, indicating the predominance of the carbohydrate component in the composition in the state of nutritional rest. The lumens of the acini were quite variable in shape. In the peripheral part of the acini, elongated nuclei of myoepithelial cells are visualized, the contraction of which ensures secretion secretion and prevents acini from stretching in case of secretion accumulation (Fig. 1).

After a week of use of the complex of food additives, the average values of the outer diameter significantly decreased by 33.67 %, which amounted to $22.04 \pm 0.20 \mu\text{m}$, the values of the inner diameter were also significantly lower by 39.74 % than the control values, and amounted to $2.76 \pm 0.08 \mu\text{m}$ ($p < 0.05$). The mean values of epithelial cell height significantly decreased by 36.07 % compared to the results of the control group, which was equal to $9.02 \pm 0.14 \mu\text{m}$ ($p < 0.05$).

The cytoplasm of the epithelial cells of the acini was heterogeneous, secretory granules of different optical density were determined, due to the presence of protein and carbohydrate components in their composition. An increase in the thickness of the interstitial connective tissue layers and marked intercellular gaps were visualized. The acini and serous cells in their composition acquired a polygonal shape. The nuclei of myoepithelial cells were elongated (Fig. 2).

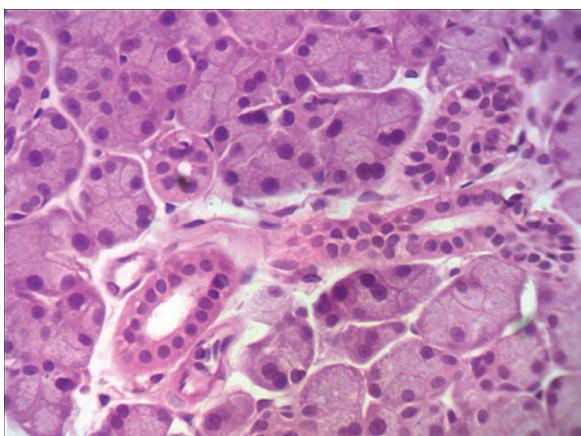


Fig. 1. The acini and excretory ducts of the submandibular salivary glands of the control group rats. Staining: Hematoxylin and eosin. Magnification: Lens x 40, Eyepiece x 10.

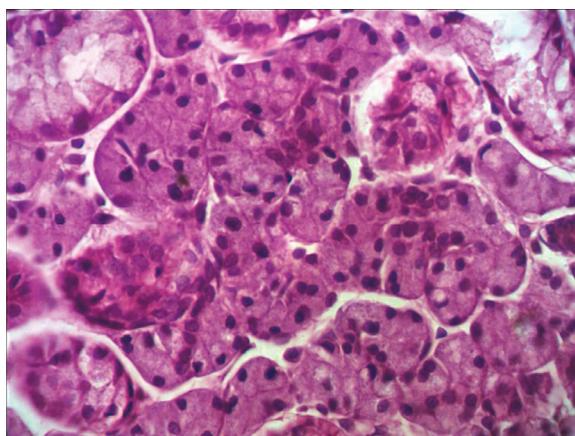


Fig. 2. Increase in the thickness of interstitial connective tissue layers on the 1st week of supplementation. Staining: Hematoxylin and eosin. Magnification: Lens x 10, Eyepiece x 40.

On the 4th week, the use of sodium glutamate, sodium nitrite and Ponceau 4R led to a significant increase in the diameter of the outer acini of the submandibular salivary glands of rats compared to the previous period of the experiment by 25.27 %, which amounted to $27.61 \pm 0.18 \mu\text{m}$, but was significantly less than that of the control group by 16.91 % ($p < 0.05$). The average values of the internal diameter were $2.64 \pm 0.07 \mu\text{m}$, which did not differ significantly from the values at week 1 of the experiment, and were significantly lower than those of the control group by 42.36 % ($p < 0.05$). The mean values of epithelial cell height were $11.96 \pm 0.11 \mu\text{m}$, which was 32.59 % significantly higher than the values of the 1st week of the experiment, and 15.24 % significantly lower than the values in the control group of animals ($p < 0.05$).

As a result of the effect of the complex of food additives on the 8th week, the average values of the external diameter significantly increased by 45.85 % compared to the previous period of the experiment, which was $40.27 \pm 0.47 \mu\text{m}$, and was also 21.19 % significantly higher than the value in the control group of animals ($p < 0.05$). The mean values of the diameter of the inner acini of the mandibular salivary glands of rats were also significantly higher than the mean values of morphometric parameters at week 4 of the experiment by 53.41 %, which amounted to $4.05 \pm 0.06 \mu\text{m}$, but were significantly lower than their value in the control group of animals by 11.57 % ($p < 0.05$). The average values of epithelial cell height significantly increased from the previous period of the experiment by 47.58 % and amounted to $17.65 \pm 0.08 \mu\text{m}$, which was also significantly higher than the control values by 25.09 % ($p < 0.05$).

At the 12th week of the experiment, when using the complex of chemicals, the average values of the diameter of the external acini were $37.60 \pm 0.53 \mu\text{m}$, which was 6.63 % significantly less than the values at the 8th week of the experiment, but 13.15 % significantly more than the values in the control group of animals ($p < 0.05$). The average values of the internal diameter significantly decreased compared to the previous period of the experiment by 61.98 % and amounted to $1.54 \pm 0.02 \mu\text{m}$, which was also by 66.38 % significantly less than in the control group of rats ($p < 0.05$). The average height of epithelial cells

significantly decreased by 17.96 % and was equal to $14.48 \pm 0.09 \mu\text{m}$, but was significantly higher by 2.62 % than in the control group of animals ($p < 0.05$).

At week 16, the complex of food additives of sodium glutamate, sodium nitrite and Ponceau-4R led to a significant increase in the average diameter of the outer acini of the submandibular salivary glands of rats by 8.32 %, compared to the previous study period, which amounted to $40.73 \pm 0.79 \mu\text{m}$, and was also significantly higher than that of the control group by 22.57 % ($p < 0.05$). The average values of the internal diameter significantly increased compared to the values at week 12 by 89.61 %, and amounted to $2.92 \pm 0.03 \mu\text{m}$, but were significantly lower than the average values in the control group of animals by 36.24 % ($p < 0.05$). The average height of the epithelial cells of the acini was significantly higher by 1.18 % than the value of the previous study period, which was also significantly higher than their values in the control group of animals by 3.83 %, which amounted to $14.65 \pm 0.14 \mu\text{m}$ ($p < 0.05$).

Microscopic examination of sections of the submandibular salivary glands of rats revealed that the cytoplasm of glandulocytes was filled with sectored granules that showed signs of increased basophilia, which, unlike the control group, determines the production of protein secretion. The nuclei of most seromucous cells were predominantly located in the basal parts of the cells and had both flattened and rounded shapes. Meanwhile, the preparations visualized areas in which the cytoplasm of epithelial cells was filled with optically bright secretory granules, with an altered shape of the acini to a tubular configuration, increased in size, cylindrical lumen shape, indicating their complete sloughing, and restructuring of the secretory apparatus in favor of carbohydrates (Fig. 3).

Thus, the use of a complex of food additives sodium glutamate, sodium nitrite and Ponceau-4R leads to changes in the morphometric parameters of the acini of the submandibular salivary glands of rats, and in the early stages of the experiment was expressed by a significant decrease in the diameter of the outer, inner diameter and height of epithelial cells (Fig. 4).

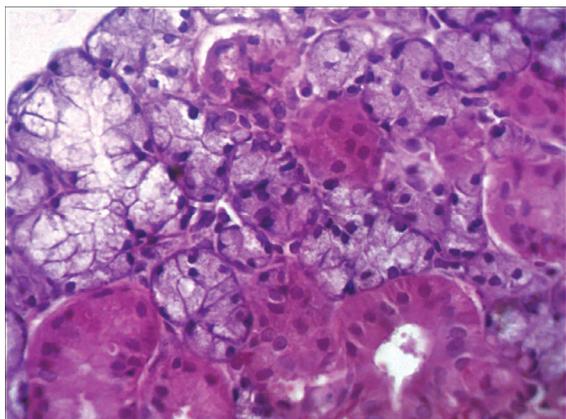


Fig. 3. Phenomena of dehiscence of the acini of the submandibular salivary glands on the 16th week of use of the complex of sodium glutamate, sodium nitrite and Ponceau-4R. Staining: Hematoxylin and eosin. Magnification: Lens x 10, Eyepiece x 40.

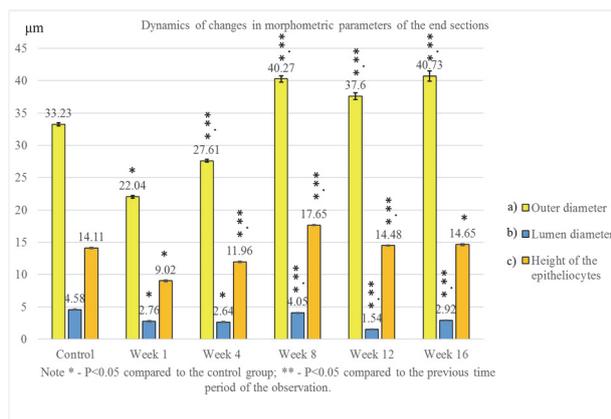


Fig. 4. Dynamics of changes in the morphometric parameters of the submandibular salivary glands of rats during the experiment: a – outer diameter, b – inner diameter, c – height of epithelial cells.

These changes are due to the primary endogenous effect of a complex of chemicals, constituents of food additives, on the state of interstitial spongiosis tissue, which indicates the initial stages of the development of hyperhydration and was confirmed by the results of histological examination (Fig. 2), which was also identified with the results of previously conducted studies on the effect of exogenous factors on tissues and organs [13, 15].

Later in the experiment, a wave-like pattern of morphometric changes in the acini of the mandibular salivary glands of rats was observed. These changes are evidently linked to compensatory and restorative responses of the body. However, complete recovery did not occur, as by week 8, a notable increase in all morphometric parameters was observed compared to those in the control group. Obviously, the complex of food additives led to changes in the secretory activity of seromucous glandulocytes during the experiment and a constant restructuring of the secretory apparatus, since an increase in secretory activity is associated with an increase in the volume of transmural fluid transport from the vessels of the microcirculatory bed to the interstitium, thus an increase in morphometric parameters is a morphological confirmation of an increase in the functional activity of seromucous cells of the salivary acini.

At the end of the experiment, the morphometric measurements revealed that the outer diameter and height of epithelial cells remained significantly elevated compared to those in the control group. This

resulted in a reduction in the inner diameter, indicative of sustained stimulation of seromucous cells. Histological analysis confirmed this, demonstrating a shift towards increased basophilia in the proteinaceous component of the acinar gland cells. Moreover, areas were identified where sloughing and reorganization of the secretory apparatus favored carbohydrates.

Conclusion

The use of a complex of food additives leads to a change in the structural and functional state of the acini of the submandibular salivary glands of rats, which was expressed by a significant decrease in the morphometric parameters of the outer diameter, inner diameter and height of epithelial cells due to the primary endogenous action of the components of the complex of food additives with an increase in the volume of the interstitium in the early stages of the experiment, but later, the action of compensatory and restorative reactions of the body did not lead to complete recovery, as there was a constant restructuring of the secretory apparatus of seromucous cells, which was confirmed by a significant wave-like increase in the diameter of the outer and height of epithelial cells, against the background of a decrease in the diameter of the lumen. The activation of secretion is associated with the reaction of the submandibular salivary glands to a change in the nature of nutrition associated with the presence of food additives and the restructuring of the base of the serotonergic apparatus in favor of proteins, with the appearance of some areas of complete sloughing, and is a morphological confirmation of the predominance of the carbohydrate component, and is protective in relation to the effect of the components of the food additives complex on the mucous membrane of both the oral cavity, and the initial parts of the gastrointestinal tract.

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