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REACTIVE CHANGES IN THE VESSELS OF THE HEMOMICROCIRCULATORY BED IN THE LOBULES OF THE SUBMANDIBULAR SALIVARY GLANDS OF RATS UNDER THE INFLUENCE OF A COMPLEX OF FOOD ADDITIVES

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The study presents morphometric data on the effects of a combination of food additives on the condition of the hemomicrocirculatory vessels in the lobules of rat submandibular salivary glands. It was found that early exposure to monosodium glutamate, sodium nitrite, and Ponceau 4R leads to narrowing of the vessels in the resistive, exchange, and capacitive segments, resulting in tissue hypoxia. This directly impairs salivary gland function and may contribute to the development of dry mouth. As part of the body's compensatory and restorative responses, dilation of the exchange segment vessels was observed, indicating intensified secretion and salivation processes. However, persistent spasms in arterioles and capillaries, along with dilation of venules, contribute to interstitial tissue edema, impaired oxygen and amino acid delivery to the terminal acinar cells, and ultimately to structural remodeling of the secretory apparatus in favor of carbohydrate over protein synthesis. This shift negatively affects saliva quality and reduces its enzymatic activity.

Key words: salivary glands, blood vessels, food additives, monosodium glutamate, sodium nitrite, Ponceau 4R, rats.

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РЕАКТИВНІ ЗМІНИ СУДИН ГЕМОМІКРОЦИРКУЛЯТОРНОГО РУСЛА ЧАСТОЧОК ПІДНИЖНЬОЩЕЛЕПНИХ СЛИННИХ ЗАЛОЗ ЩУРІВ ПІД ВПЛИВОМ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК

В роботі представлені дані морфометричного дослідження при комплексній дії харчових добавок на стан судин гемомікроциркуляторного русла часточок піднижньощелепних слинних залоз щурів. Встановлено, що при дії глутамату натрію, нітриту натрію та Понсо 4R на ранніх термінах спостереження спостерігається звуження судин резистивної, обмінної та ємнісної ланок з розвитком гіпоксії тканин, що безпосередньо впливає на функцію слинних залоз та може призводити до сухості ротової порожнини. Внаслідок компенсаторно-відновлювальних реакцій організму, спостерігається розширення судин обмінної ланки, що свідчить про посилення процесів секретоутворення та слиновиділення. Однак, спастичні явища в артеріолах і капілярах, разом з розширенням венул, призводять до розвитку набрякових явищ в інтерстиційній тканині, порушення надходження кисню та амінокислот до клітин кінцевих відділів, і, як наслідок, перебудовою секреторного апарату на користь вуглеволів, що погіршує якість слини та знизить її ферментативну активність.

Ключові слова: слинні залози, судини, харчові добавки, глутамату натрію, нітриту натрію, Понсо 4R, щурі.

The study is a fragment of the research project "Structural remodeling of the organs of the immune, respiratory, and excretory systems under the influence of various exogenous factors (monosodium glutamate, sodium nitrite, ethanol, methacrylate)," state registration No. 0121U108234.

As is well known, the condition of the hemomicrocirculatory bed plays a significant role in the functioning of tissues and organs, serving as a vital prerequisite for maintaining their normal activity.

The intensity of secretion by glandulocytes in the salivary glands is directly dependent on the level of their blood supply. This is closely linked to the response of blood vessels and involves the filtration of fluid, as well as organic and inorganic substances, through the glandular epithelium. As a result, the terminal acinar cells produce the protein and carbohydrate components of saliva. Therefore, studying the morphofunctional characteristics of the salivary glands requires reliable data on the structural features of the hemomicrocirculatory bed, taking into account specific functional regions [13].

Recent scientific publications have examined the effects of various food additives on organs and physiological systems. However, current data remain insufficient, and studies investigating their combined effects remain scarce [1, 2, 3].

The most well-known and widely used flavor enhancer is E-621 – monosodium glutamate (MSG), which intensifies taste perception but also exhibits pathophysiological and toxicological effects. Numerous studies by international researchers highlight its harmful impact [7, 9, 15]. As a result, there is currently no consensus on a safe dosage of this commonly used food additive. Furthermore, the mechanisms underlying the pathogenic and damaging effects of monosodium glutamate remain insufficiently studied.

In Ukraine, food additive E-250 (sodium nitrite) is commonly used as a color fixative in the production of meat products [5]. It has been reported that sodium nitrite exerts harmful toxic effects on

various organs. According to a study by Ukrainian researchers, chronic exposure to sodium nitrite induces oxidative stress (evidenced by elevated levels of 2,3-bisphosphoglyceric acid), inflammation (increased levels of interleukin-1 beta, which in turn triggers a sharp rise in iNOS activity), and endothelial dysfunction (elevated levels of von Willebrand factor) [12].

Food additive E-124 is used to enhance the visual appeal of products [10], but like many synthetic colorants, it poses health risks. Its hazardous effects are associated with its metabolic by-product, benzidine – a compound known to induce various types of tumors in both humans and animals. Another component of the azo dye, p-phenylenediamine, is a known contact allergen [6].

The purpose of the study was to determine the dynamics of morphometric changes in the lumen diameter of hemomicrocirculatory vessels in the submandibular salivary glands of rats under normal conditions and under the combined influence of food additives – monosodium glutamate, sodium nitrite, and Ponceau 4R.

Materials and methods. The study involved 84 sexually mature male rats. Animals in the control group were given drinking water and received oral administration of physiological saline. Rats in the experimental group, with free access to water, were orally administered a combination of food additives once daily: sodium nitrite at a dose of 0.6 mg/kg, monosodium glutamate at 20 mg/kg, and Ponceau 4R at 5 mg/kg, all dissolved in 0.5 ml of distilled water. These doses were half of the maximum permissible limits. Adaptive behavior of the rats was assessed using the open field test. Animals were euthanized at 1, 4, 8, 12, and 16 weeks by an overdose of thiopental anesthesia. After euthanasia, fragments of the submandibular salivary glands were fixed in 10 % formalin. The tissue samples were then embedded in paraffin using standard histological procedures [11]. Sections of 5-10 μm thickness were prepared using an ARM 3600 microtome. After staining with hematoxylin and eosin, the sections were mounted in polystyrene and examined under a light microscope. Microphotography and morphometric analysis were performed using a Levenhuk D740T digital microscope equipped with a digital photoadapter and software adapted for this study. Statistical analysis of the morphometric data was conducted using Microsoft Excel with the built-in "Analysis ToolPak – VGA" add-on, specifically the "Descriptive Statistics" tool. The Shapiro–Wilk test was used to assess normality of the data distribution, and for normally distributed variables, comparisons were made using the Student's t-test for independent samples. Differences were considered statistically significant at $p < 0.05$ [4, 8].

All animal experiments were carried out in compliance 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 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 analysis of the lumen diameter of hemomicrocirculatory vessels in the submandibular salivary glands revealed that, in rats of the control group, the mean arteriolar lumen diameter was 12.51 ± 0.01 μm, the diameter of capillaries measured 4.23 ± 0.04 μm, and the diameter of venules was 16.58 ± 0.05 μm (Table 1).

Table 1

Morphometric parameters of the lumen diameter of hemomicrocirculatory vessels in the lobules of rat submandibular salivary glands

Parameters	Lumen diameter of intraclobular vessels of the hemomicrocirculatory bed		
	Diameter of arteriole lumen (μm)	Diameter of capillary lumen (μm)	Diameter of venule lumen (μm)
Control	10.80±0.07	4.98±0.09	13.21±0.13
1 week	9.58±0.11 *	3.05±0.07 *	11.91±0.08 *
4 weeks	8.94±0.11 *,**	5.17±0.07 *,**	12.02±0.05 *
8 weeks	6.10±0.08 *,**	3.24±0.06 *,**	15.08±0.18 *,**
12 weeks	8.47±0.11 *,**	3.83±0.30 *,**	13.61±0.07 *,**
16 weeks	8.60±0.17 *	3.94±0.17 *	15.22±0.47 *,**

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

The administration of food additives during the first week of the experiment resulted in a significant decrease in the mean lumen diameter of arterioles by 11.30 % ($p < 0.05$), measuring 9.58 ± 0.11 μm.

Capillary lumen diameter decreased by 38.76 % ($p < 0.05$), reaching 3.05 ± 0.07 μm. Venule lumen diameter also reduced by 9.84 % ($p < 0.05$), with an average value of 11.91 ± 0.08 μm ($p < 0.05$).

Histological examination of the hemomicrocirculatory vessels in the lobules of rat submandibular salivary glands at the first week revealed structural changes in the arteriole walls. Under the influence of the combination of monosodium glutamate, sodium nitrite, and Ponceau 4R, the intimal layer consisted of a layer of endothelial cells whose nuclei protruded into the vessel lumen. In the medial layer, disruptions of the contacts between smooth muscle cells were observed. In the vessels of the capacitive segment, a thinning of the adventitial layer was noted, and signs of lumen obstruction were present (Fig. 1).

By the 4th week of the experiment, vessels of the resistive segment showed a significant decrease in mean lumen diameter by 6.68 % compared to the previous observation period, measuring 8.94 ± 0.11 μm. This value was also significantly lower by 17.22 % compared to the control group ($p < 0.05$). The vessels of the exchange segment responded with a significant increase in lumen diameter by 69.51 % compared to the 1st week of the experiment, which was also 16.08 % greater than the control group values ($p < 0.05$). The mean capillary lumen diameter at week 4 was 5.17 ± 0.07 μm. For the capacitive segment, the mean lumen diameter increased to 12.02 ± 0.05 μm, which was not significantly different from the previous time point but was significantly lower than the control group by 9.01 % ($p < 0.05$).

By the 8th week, exposure to the combination of monosodium glutamate, sodium nitrite, and Ponceau 4R caused a significant reduction in the mean arteriole lumen diameter by 31.77 % compared to week 4, measuring 6.10 ± 0.08 μm. This value was also significantly lower by 43.52 % than that of the control group ($p < 0.05$). Morphometric measurements showed a significant decrease in capillary lumen diameter both compared to the previous observation period (37.33 %) and the control group (34.94 %), with a value of 3.24 ± 0.06 μm ($p < 0.05$). Venules at week 8 exhibited sustained dilation, confirmed by a significant increase in mean lumen diameter to 15.08 ± 0.18 μm. This was 25.46 % greater than at the previous time point and 14.16 % larger than the control group values ($p < 0.05$) (Fig. 2).

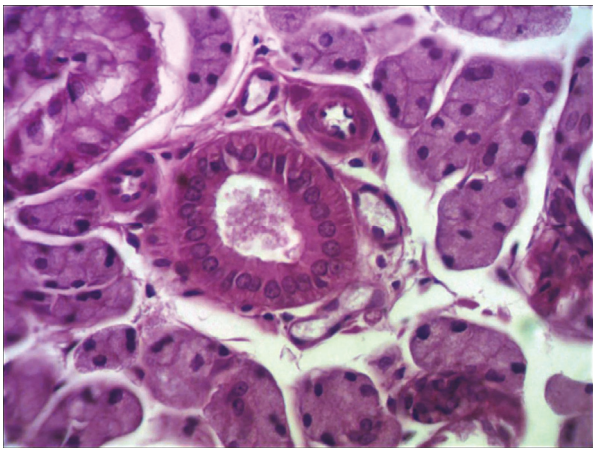

Fig. 1. Narrowing of resistive vessels accompanied by signs of lumen obliteration in capacitive vessels of rat submandibular salivary glands after 1 week of exposure to a combination of monosodium glutamate, sodium nitrite, and Ponceau 4R. Hematoxylin and eosin staining. Magnification: oc.10, ob.40.

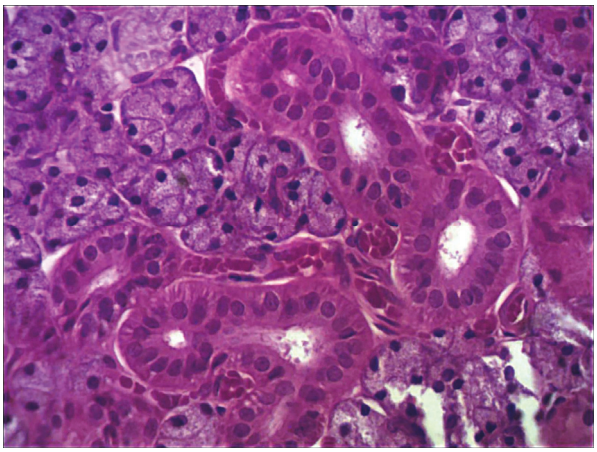

Fig. 2. Dilation with signs of blood engorgement in the venules of rat submandibular salivary gland lobules after 8 weeks of exposure to the food additive complex. Hematoxylin and eosin staining. Magnification: oc.10, ob.40.

By the 12th week of the experiment, the mean morphometric values of arteriole lumen diameter measured 8.47 ± 0.11 μm, representing a significant increase of 38.85 % compared to the previous observation period, but remained significantly lower than the control group by 21.57 % ($p < 0.05$). The mean capillary lumen diameter significantly increased to 3.83 ± 0.30 μm, which was 18.21 % greater than at week 8 but still significantly lower than in the control group by 23.09 % ($p < 0.05$). Venule lumen diameter significantly decreased by 9.74 % compared to week 8 but was significantly greater than the control group by 3.03 % ($p < 0.05$). The mean venule diameter at week 12 was 13.61 ± 0.07 μm.

At the 16th week, exposure to the food additive complex resulted in an increase in the mean arteriole lumen diameter to 8.60 ± 0.17 μm, which was not significantly different from the previous time point but remained 20.37 % smaller than that of the control group ($p < 0.05$). The lumen diameter of the exchange segment vessels significantly increased by 2.87 % compared to the previous time point but remained significantly lower than the control group by 20.88 % ($p < 0.05$). The mean capillary lumen diameter was 3.94 ± 0.17 μm. The capacitive segment vessels showed a significant increase in lumen diameter, with mean values of 15.22 ± 0.47 μm, which was significantly greater than both the 12th-week measurements by 11.83 % and the control group by 15.22 % ($p < 0.05$).

Thus, the combined action of monosodium glutamate, sodium nitrite, and Ponceau 4R leads to changes in the morphofunctional structure of the hemomicrocirculatory vessels in the submandibular salivary glands of rats. At early stages, this is manifested by a reduction in the mean lumen diameters of the resistive, exchange, and capacitive vessel segments. This response is evidently related to the primary endogenous effects of these substances on the submandibular salivary gland, whose vessels reacted with lumen narrowing, reflected in a significant decrease in the mean lumen diameters of arterioles by 11.30 %, capillaries by 38.76 %, and venules by 9.84 % ($p < 0.05$) (Fig. 3).

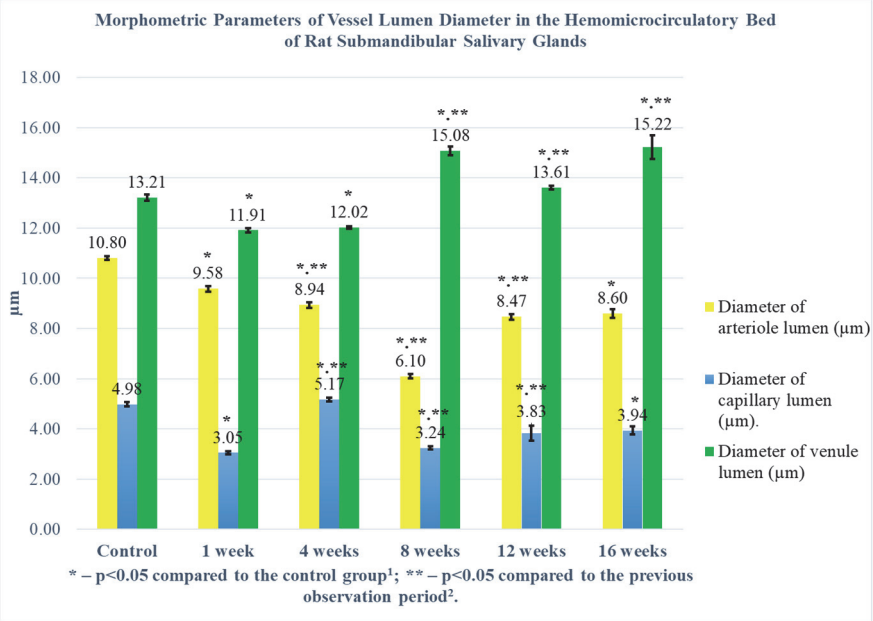

Fig. 3. Graph illustrating the dynamic changes in the lumen diameter of vessels in the lobules of rat submandibular salivary glands throughout the experiment.

Naturally, such a reaction led to hypoxia in the glandular tissue and inevitably affected the primary function of the salivary glands, namely saliva production and secretion, which likely resulted in dry mouth. Subsequently, in response to the combined action of monosodium glutamate, sodium nitrite, and Ponceau 4R, compensatory and adaptive reactions of the organism to these food additives were observed, manifested by significant dilation of the exchange segment vessels, which showed a 69.51 % increase in lumen diameter. This occurred against the background of venule narrowing, with their mean lumen diameter significantly reduced by 9.01 % compared to the control group ($p < 0.05$), indicating an enhancement of secretory and salivary flow processes. However, these adaptive and restorative responses are temporary, as from the 8th week of the experiment onwards, a significant reduction in the lumen diameter of resistive and exchange vessels and dilation of capacitive vessels led to the development of edema in the interstitial tissue of the submandibular salivary glands in rats. Spastic phenomena in arterioles and capillaries further reduced the delivery of oxygen and essential amino acids to the seromucous cells of the terminal portions, causing a remodeling of the secretory apparatus towards carbohydrate production, with the appearance of mucous foci (Fig. 4).

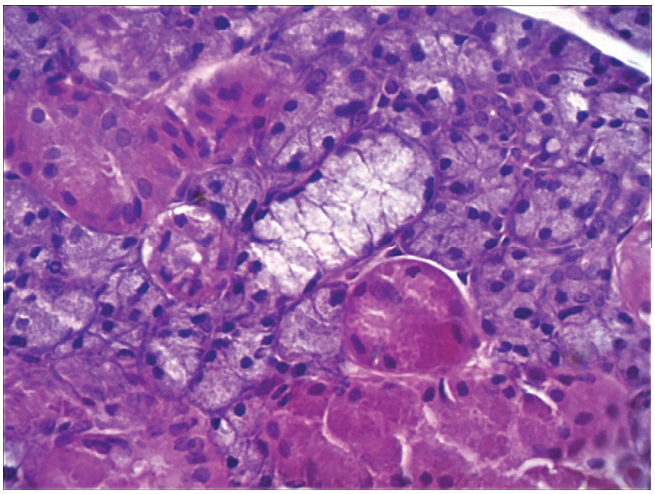

Fig. 4. Mucous metaplasia in the terminal portions of lobules of the submandibular salivary glands in rats from the experimental group after 16 weeks of exposure to the food additive complex. Hematoxylin and eosin staining. Magnification: oc.10, ob.40.

This behavior can be compared to the effects of other exogenous factors previously studied by us, such as ethanol, which, when acting on the submandibular salivary gland, caused narrowing of the hemomicrocirculatory vessels at early stages of the experiment, demonstrating their direct impact on the vascular bed [13], as well as other exogenous agents affecting various organs and tissues [11].

This finding is consistent with previous studies on the effects of the food additive complex on the terminal portions of rat submandibular glands [14]. Thus, the reactive changes in the vessels of the hemomicrocirculatory bed under the combined influence of food additives exhibit antagonistic characteristics and may be a causative factor in the development of xerostomia.

Conclusion

Thus, the combined action of food additives – monosodium glutamate, sodium nitrite, and Ponceau 4R – induces changes in the morphofunctional state of the hemomicrocirculatory vessels in the submandibular salivary glands of rats. In the early stages of exposure, narrowing of the vessels in the resistive, exchange, and capacitive segments is observed, leading to tissue hypoxia. This directly affects the functional activity of the salivary glands and may contribute to the development of dry mouth. As part of the body's compensatory and restorative responses, dilation of the exchange segment vessels occurs, indicating enhanced processes of secretion and salivation. However, the persistence of vasospastic phenomena in arterioles and capillaries, combined with venular dilation, results in the development of interstitial edema, impaired delivery of oxygen and amino acids to the acinar cells, and, consequently, a shift in the activity of the secretory apparatus toward carbohydrate production. This shift compromises saliva quality and reduces its enzymatic activity.

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