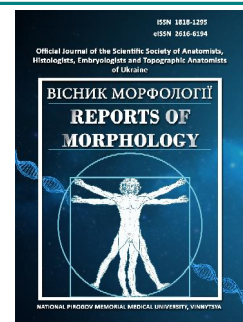




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# Structural organization of the carotid sinus under the influence of monosodium glutamate in the experiment: analysis of changes in dynamics

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### CONFLICT OF INTEREST

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Carotid artery pathology is one of the leading causes of cerebral stroke. Among the pathogenetic factors in the development of carotid artery damage, disorders of lipid metabolism, atherosclerosis, and metabolic syndrome occupy a prominent place. The alimentary factor is extremely important in this context. Monosodium glutamate is one of the most common food additives, which is often used uncontrollably and can cause changes in the structure and functions of organs and tissues. The purpose of the study: to analyze the dynamics of morphological changes in the carotid sinus area under the influence of monosodium glutamate when administered orally in an experiment. The area of the carotid sinus of 20 male laboratory white rats that received sodium glutamate orally at a dose of 10 mg/kg/day for 8 weeks was studied by morphological methods at the macro- and microstructural levels after 6 and 8 weeks of the experiment. The obtained data are compared with the results of a morphological study of the same area in 20 animals of the control group. Statistical processing of animal weight was performed using MS Excel 2007 software. Mean  $\pm$  standard deviation was determined. After 6 weeks of the experiment, when evaluating the histological structure of the wall of the internal carotid artery in the area located directly above the bifurcation, when compared with the control group, multiplication and folding of the intima were found in the experimental group, presumably associated with the proliferation of endothelial cells under the influence of sodium glutamate, detachment of the endothelium and lysis of individual endotheliocytes, as well as uneven thickening of elastic media fibers and disruption of their structure. Attention was drawn to the accumulation of white fat perivasally and in the zone of the carotid glomus, as well as the disorganization of nerves and the expansion of vessels of the microcirculatory channel. After 8 weeks of the experiment, the negative dynamics of structural changes were noticeable: signs of increased inflammatory infiltration, deformation of the vessels of the microcirculatory bed with thickening of their walls and narrowing of the lumen, stasis, noticeable degranulation of cells of type I carotid glomus cells, the appearance of single labrocytes (mast cells) in the infiltrate. The amount of adipose tissue (white fat) in the area of the carotid sinus and the perivascular bifurcation of the carotid arteries, as well as in the immediate vicinity of the carotid glomus, also increased markedly, and a tendency towards thickening of adipose tissue was noted. Thus, monosodium glutamate with systematic oral use can cause a violation of the structural organization of the carotid sinus, the wall of the carotid arteries and the carotid glomus, and the severity of changes in dynamics increases. Further research is needed to clarify the nature of the structural changes in the carotid sinus under the conditions of withdrawal of monosodium glutamate, as well as to find possible ways of correction.

**Keywords:** monosodium glutamate, carotid sinus, carotid glomus, internal carotid artery, carotid bifurcation.

### Introduction

Cardiovascular diseases, in particular myocardial infarction and cerebral stroke, continue to hold the leadership

in the structure of the causes of mortality in the whole world, and also occupy a prominent place in the statistics of

premature deaths (up to 70 years of age) according to the data of the World Health Organization [19]. One of the leading causes of cerebral blood circulation disorders, including cerebral strokes, is the pathology of the carotid arteries [5, 8]. An important role in its development is played not only by a family predisposition to dyslipidemia, but also completely controllable factors - a sedentary lifestyle, unhealthy diet, in particular, the consumption of high-calorie foods with a significant fat content, as well as the widespread use in the food industry of additives to improve the organoleptic properties of food [19]. The most common among them is monosodium glutamate, also known as additive E621 or monosodium salt of glutamic acid, which is considered relatively safe and is allowed for use in many countries of the world, in particular in Ukraine. Additive E621 is used to enhance the taste and improve the organoleptic properties of food, which are lost due to long-term storage [15].

Despite numerous studies reporting the harmful effects of monosodium glutamate on the living organism [1], its use continues to be widespread and sometimes uncontrolled due to the fact that the presence of the additive E621 is often not even recorded on food packaging. The monosodium salt of glutamic acid is also present in baby food products. Since it was reported about the effect of the E621 supplement on brain tissue [6], the human psyche [11], as well as the ability to cause changes in eating behavior, manifested in increased appetite and an increase in the amount of food consumed, with the subsequent development of excess body weight and obesity [2, 17], the long-term administration of monosodium glutamate is a cause for concern, given the potential adverse effects that can be expressed in pathological changes in the structure of organs and tissue and subsequently lead to a violation of their functions and the development of a number of pathological conditions.

Oxidative stress is considered one of the important pathogenetic aspects of sodium glutamate's influence on a living organism [6, 7]. An important role is also played by the development of inflammation and fibrosis [7], metabolic disorders [3, 18] and DNA damage - the so-called genotoxic effect [1, 10]. Numerous studies have reported adverse effects of monosodium glutamate on the heart [3] and vessels, including the aorta [3] and thoracic arteries [13], as well as other organs [4]. Given the important role of the morphology of the carotid arteries in the pathogenesis of brain perfusion disorders, the effect of the systematic use of monosodium glutamate on their structure has not been sufficiently studied. Of particular interest is the nature of morphological changes, their dynamics and the degree of irreversibility of possible damage, the reaction of tissues to the withdrawal of monosodium glutamate, as well as the possibility of correction [14].

*The purpose of the study* is to analyze the dynamics of morphological changes in the carotid sinus area under the influence of monosodium glutamate when administered orally in an experiment.

## Materials and methods

40 male white laboratory rats were involved in the study, which were equally divided into experimental and control groups. The animals were kept in cages of 4 individuals each, in a well-ventilated room of the vivarium, and had unlimited access to food corresponding to the standard diet of the vivarium. Animals of the experimental group received 10 mg/kg/day of monosodium glutamate administered orally for 8 weeks, while animals of the control group did not receive food supplements. Throughout the experiment, the principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the norms of Law of Ukraine № 3447-IV "On the Protection of Animals from Cruelty Treatment", general ethical principles of experiments on animals, adopted by the First National Congress of Ukraine on Bioethics (2001), which was confirmed by the Commission on the Ethics of Scientific Research, Experimental Developments and Scientific Works of Danylo Halytsky Lviv National Medical University (protocol № 3 dated 11.3.2022) were strictly observed.

After 6 weeks from the start of the study, half of the animals were removed from the experiment by overdose of ether anesthesia; the rest of the animals were removed from the experiment at the end of the 8th week. The research material is represented by histological preparations of the carotid sinus of white rats, made by making sections of the tissue of the bifurcation of the carotid arteries from previously prepared paraffin blocks. For histological examination, sections were stained with methylene blue. The preparations were studied and photographed at magnifications of the microscope: x200, x400, x1000. The "Aver Media" computer system was used to photograph micropreparations.

Statistical processing of animal weight was performed using MS Excel 2007 software (mean  $\pm$  standard deviation was determined).

## Results

The average weight of animals in the control group after 6 and 8 weeks of the experiment was 276.5 $\pm$ 6.3 g and 283.5 $\pm$ 5.6 g, in the experimental group this indicator was 337.4 $\pm$ 4.3 g and 364.5 $\pm$ 6.2 g, respectively.

During the morphological study of the studied area of the carotid sinus of a white rat, it was found that the internal and external carotid arteries originated from the common carotid artery. The bifurcation of the common carotid artery was typically located at the posterior angle (greater horn) of the hyoid bone, 2-4 mm below the latter in animals of both groups. The diameter of the internal carotid artery practically corresponded to that of the external carotid artery. The bifurcation of the carotid arteries in animals of both groups was located below the typical location in 17.5 % of cases (7/40 adult white rats). The location of nerves and sensory nerve endings in the carotid sinus and carotid glomus in rats of both groups was similar to that in humans.

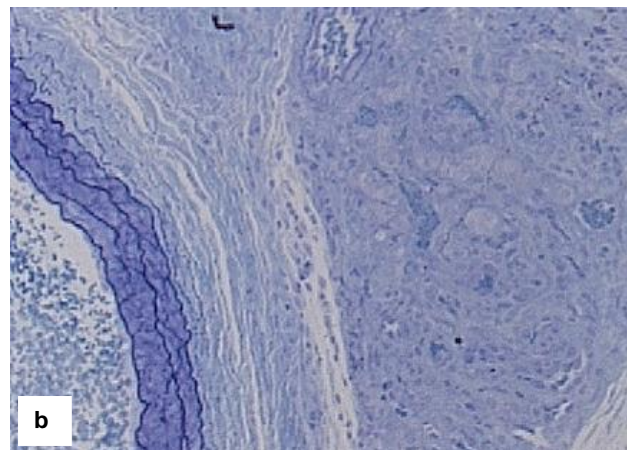
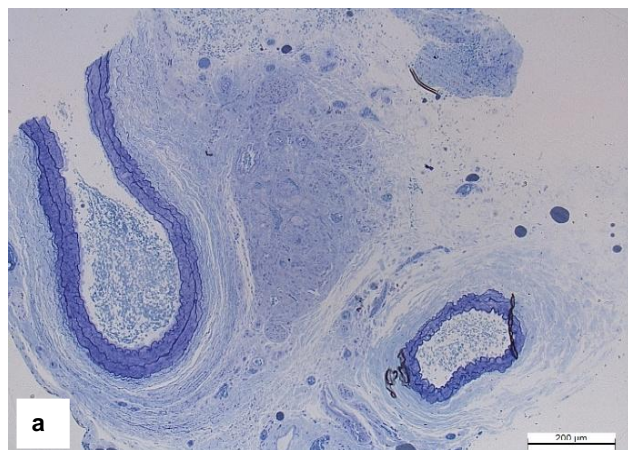
The carotid glomus was located in the carotid sinus, closer to the internal carotid artery, and had a size of 0.6-0.7 mm by 0.5-0.4 mm, and was surrounded by connective tissue. Its vascularization occurred due to a small branch of the external carotid artery - the glomus artery, and venous outflow - into a small vein of the same name, which opened into the internal jugular vein. Sensory innervation was provided by branches of the glossopharyngeal and vagus nerves, autonomic innervation by fibers of parasympathetic nodes, in which the branches of the vagus nerve are interrupted, as well as by the sympathetic plexus, and the sympathetic trunk was located dorsal to the common carotid artery and the vagus nerve. Cervical nodes in this area were grouped into three pairs: upper, middle, and posterior. The upper cervical node lay at the level of the bifurcation of the common carotid artery in close proximity to the internal carotid artery, carotid sinus, and carotid glomus.

During histological examination (staining with methylene blue), the wall of both carotid arteries consisted

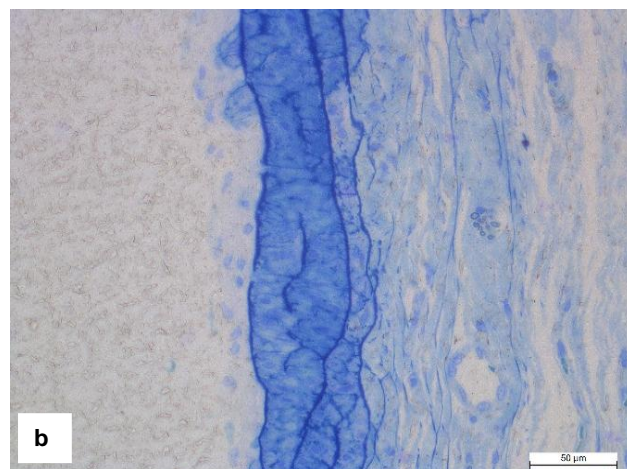
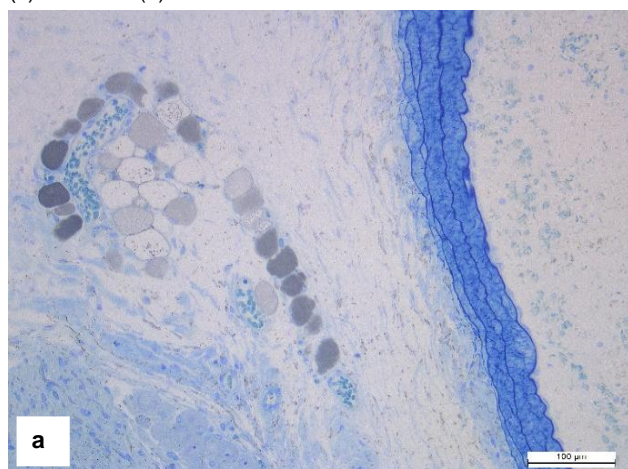
of clearly defined three layers: inner - intima, middle - media, and outer - adventitia, in which small blood vessels, known as vasa vasorum, were visible.

After 6 weeks of the experiment, when evaluating the histological structure of the wall of the internal carotid artery in the area located directly above the bifurcation, when compared with the control group (Fig. 1), in the experimental group (Fig. 2), multiplication and folding of the intima, presumably associated with proliferation, were found of endothelial cells under the influence of monosodium glutamate, detachment of the endothelium and lysis of single endotheliocytes, as well as uneven thickening of the elastic fibers of the media and disruption of their structure, which causes changes in the intima-media ratio and may ultimately have a negative effect on the perfusion of brain tissues. Attention was drawn to the accumulation of white fat perivascularly and in the zone of the carotid glomus, as well as the disorganization of nerves and the expansion of vessels of the microcirculatory channel.

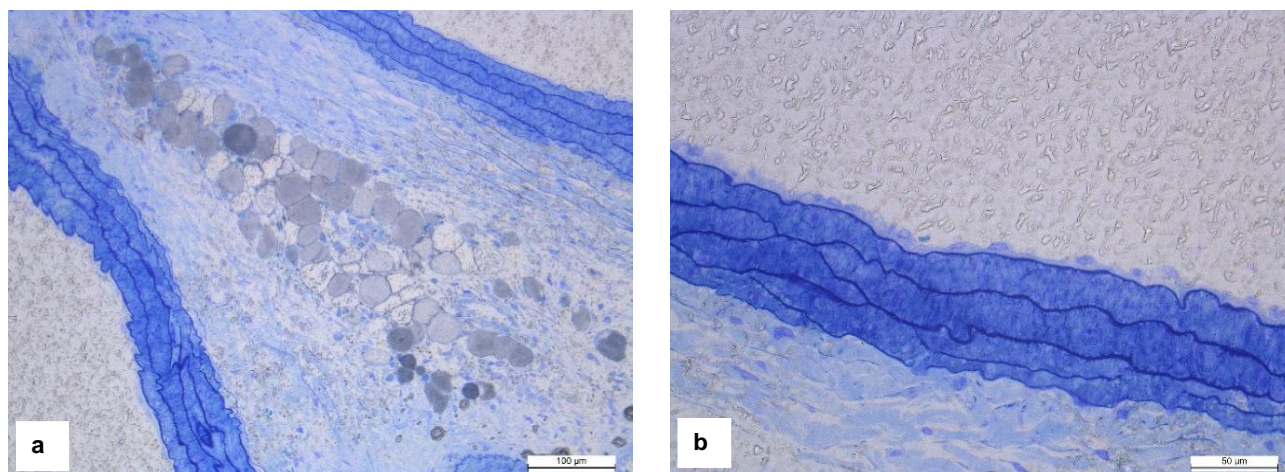
After 8 weeks of the experiment, the histological



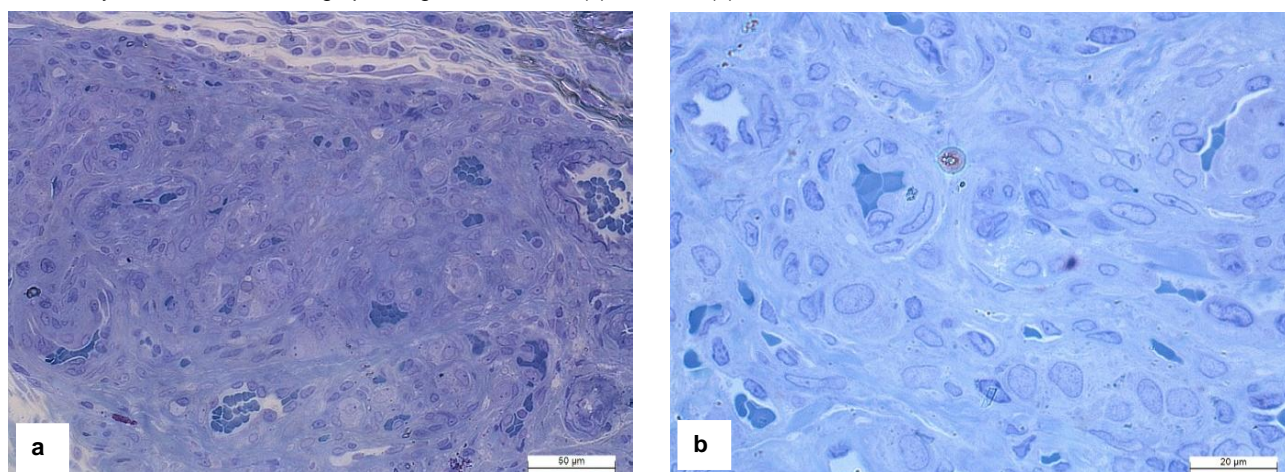
**Fig. 1.** Carotid artery bifurcation area (a) and a fragment of the internal carotid artery wall in the area immediately above the bifurcation of the common carotid artery of a white rat (b) of the control group. Staining with methylene blue. Photomicrograph. Magnification: x200 (a) and x400 (b).



**Fig. 2.** Bifurcation area of carotid arteries with carotid glomus (a) and a fragment of the wall of the internal carotid artery in the area located directly above the bifurcation of the common carotid artery of a white rat (b) of the experimental group 6 weeks after the start of the experiment. Staining with methylene blue. Photomicrograph. Magnification: x200 (a) and x400 (b).



**Fig. 3.** Carotid artery bifurcation zone (a) and a fragment of the wall of the internal carotid artery in the area located directly above the bifurcation of the common carotid artery of a white rat (b) of the experimental group 8 weeks after the start of the experiment. Staining with methylene blue. Photomicrograph. Magnification: x200 (a) and x400 (b).

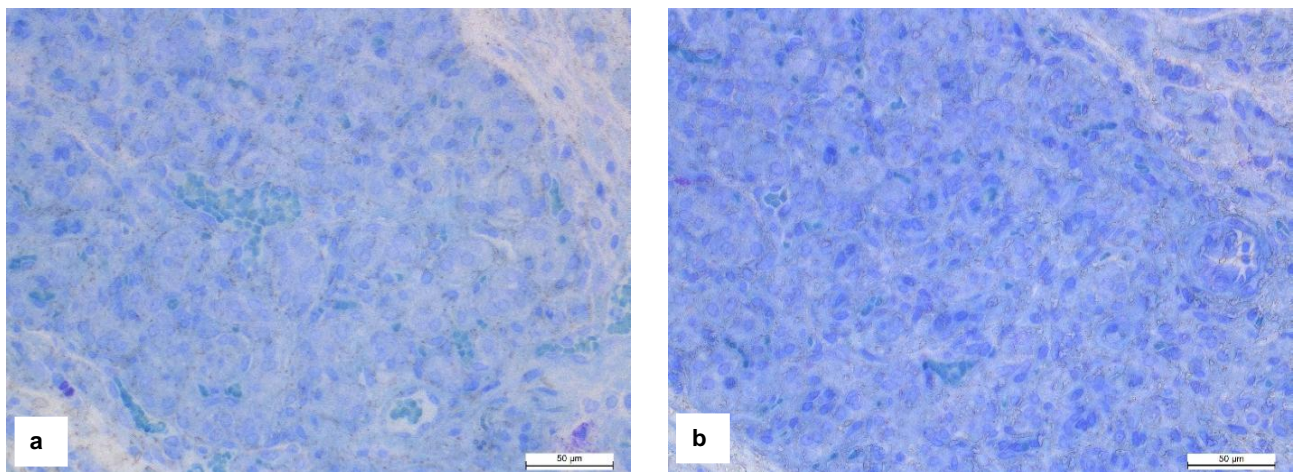


**Fig. 4.** Fragments of the glomus of a white rat of the control group. Staining with methylene blue. Photomicrograph. Magnification: x200 (a) and x400 (b).

structure of the wall of the carotid artery (Fig. 3) was characterized by an increase in the amount of adipose tissue perivasally and in the bifurcation zone of the carotid artery and the carotid glomus, the appearance of an inflammatory cell infiltrate, proliferation of arterioles, an increase in the irregularity of the vascular wall, especially expressed in the media zone, changes from the side of the connective tissue, in particular its swelling and unclear structure, hyperemia of the vessels of the microcirculatory channel.

Regarding the localization of the carotid glomus, it was located in the area of the internal carotid artery, directly above the bifurcation of the common carotid artery, 1-1.5 mm more cranially than the latter. The presence of the adventitial capillary plexus in the zone of the carotid glomus attracted attention. When staining with methylene blue, baroreceptors were detected in the form of rounded nerve endings. Four main components were identified in the carotid glomus: cells, nerve fibers, vessels and the main substance of connective tissue. A cluster of cells, also

called glomeruli, is the main structural element of the carotid glomus and consisted of two types of cells in the white rat: cells of type I (glomus cells) - from 2 to 12 cells in each glomerulus, on average 4, surrounded by cells of type II (supporting) - 1-3 cells in a glomerulus. These two cell types can be clearly distinguished even by light microscopy (Fig. 4). Glomus cells were chemoreceptors and contained secretory granules, had a round or oval shape, and their size varied from 8 to 16 μm. The nucleus of glomus cells is clearly delineated, oval in shape, the cytoplasm is granular. Type II cells, the number of which was 15-20 % of all glomus cells, were usually visible on the periphery of the clusters, resembled neuroglia cells in structure, had elongated hyperchromic nuclei, a thin cytoplasmic strip, and pronounced processes that surrounded the glomus cells. The carotid glomus also contained autonomous microganglionic cells located on the periphery of the glomus or directly in the latter. Clusters of cells are separated by layers of connective tissue, which, when connected, formed the capsule of the carotid glomus. The



**Fig. 5.** Fragments of the glomus of a white rat of the experimental group after 6 weeks (a) and 8 weeks (b) of the experiment. Staining with methylene blue. Photomicrograph. Magnification: x400.

stroma around the lobes of the carotid glomus of white rats is rich in blood vessels and nerves.

As for the structural organization of the carotid glomus, after 6 weeks of the experiment, in the control group (see Fig. 4) its typical structure was observed: type I cells were located in the glomeruli and surrounded by type II cells, between the glomeruli there were visible layers of connective tissue, then as in the experimental group, after 6 weeks of the experiment (Fig. 5a), attention was drawn to a decrease in the number of type I cells in some clusters, as well as a pronounced thickening and swelling of the connective tissue layers between them, signs of inflammation, thrombus formation, and hyperemia of the microcirculatory vessels. After 8 weeks of the experiment (Fig. 5b), the negative dynamics of structural changes were noticeable: signs of increased inflammatory infiltration, deformation of vessels of the microcirculatory bed with thickening of their walls and narrowing of the lumen, stasis, noticeable degranulation of type I cells, appearance of single labrocytes (mast cells) in the infiltrate. The amount of adipose tissue (white fat) in the area of the carotid sinus and the perivascular bifurcation of the carotid arteries, as well as in the immediate vicinity of the carotid glomus, also increased markedly, and a tendency towards thickening of adipose tissue was noted.

### Discussion

The pathology of the carotid arteries plays a leading role in the pathogenetic mechanisms of the development of cerebrovascular disorders, which lead not only to cerebral strokes, but also to cognitive changes [12]. The determination of risk factors that directly affect the morphology and, as a result, the function of the carotid arteries is of great clinical importance, since the modification of such factors allows to reduce the risks associated with morbidity, immediate and remote negative consequences for patients, loss of their working capacity, and a decrease in quality and a decrease in life expectancy.

To study the influence of external factors on the structure and function of the carotid arteries, in particular the carotid sinus, the bifurcation zone and related structures, it is advisable to use experimental models that offer wide opportunities for analyzing processes and changes, as well as factors that can modify their course [10].

This study used a low dose of monosodium glutamate that was approved for use in food in most countries. The oral route of administration followed by free access to food was expedient in order to accurately model the route of entry of monosodium glutamate into the human body during life and consumption of usual food products. It is this fact that allows the extrapolation of the experimental study to the general human population, because parenteral or subcutaneous administration of monosodium glutamate does not occur under normal conditions. An important aspect was the assessment of the morphological changes of the studied area in dynamics, as this indirectly makes it possible to predict the speed and severity of pathological changes of the studied area with systematic consumption of monosodium glutamate.

Therefore, after 6 and 8 weeks of the experiment, histological examination revealed significant changes in the structure of the wall of the carotid arteries in the carotid sinus area at the microscopic level, as well as changes in the morphology of the carotid glomus in the animals of the experimental group, which could be directly caused by the oral administration of monosodium glutamate in comparison with a control group that did not receive a food supplement. The progressive accumulation of adipose tissue perivascularly in the area of bifurcation of carotid arteries and carotid glomus attracted special attention. The growth of structural and inflammatory changes in dynamics seems to be important, which may indicate an increase in the toxic effects of monosodium glutamate over time with its systematic use with food [7]. The above-mentioned morphological manifestations could be not only a consequence of the direct toxic effect of monosodium

glutamate, but also the result of its indirect effect, namely the stimulation of the appetite of the animals of the experimental group, which led to an increased feeling of hunger, consumption of more food and, as a result, faster weight gain.

Given the key role of vascular pathology in the development of cerebral stroke, possible violations of the structural organization of blood vessels, in particular, carotid arteries, as well as areas of the carotid sinus should be taken into account in persons who systematically consume monosodium glutamate as a food additive for the possible timely prevention of unfavorable distant consequences for cerebral and systemic blood circulation by modifying risk factors and using appropriate preventive measures [16]. Preventing the development of structural changes in the carotid glomus is also of great practical importance, as a violation of the morphology will inevitably lead to a negative impact on its functions, which can potentially affect not only the quality of brain perfusion, but also the tone of blood vessels, blood pressure, and systemic blood circulation in general. The role of mast cells in conditions of structural

disorders of the carotid sinus and carotid glomus requires additional research, since the available data on the influence of the content of their granules on the functions of the body are somewhat contradictory and require clarification. The problem of the dynamics of structural changes in the carotid sinus zone under the conditions of sodium glutamate withdrawal is also of great interest, since their reversible nature can have a beneficial effect on at least partial restoration of the functions of damaged structures.

Further research is needed to clarify the nature of the structural changes in the carotid sinus under the conditions of withdrawal of monosodium glutamate, as well as to find possible ways of correction.

### Conclusion

Monosodium glutamate with systematic oral use can cause a violation of the structural organization of the carotid sinus, the wall of the carotid arteries and the carotid glomus, and the severity of the changes in dynamics increases.

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#### СТРУКТУРНА ОРГАНІЗАЦІЯ СОННОЇ ПАЗУХИ ПІД ВПЛИВОМ ГЛУТАМАТУ НАТРІЮ В ЕКСПЕРИМЕНТІ: АНАЛІЗ ЗМІН В ДИНАМІЦІ

**Содомора О. О.**

*Патологія сонних артерій є однією з провідних причин мозкового інсульту. Серед патогенетичних факторів розвитку ушкоджень сонних артерій чільне місце посідають розлади ліпідного обміну, атеросклероз і метаболічний синдром. Аліментарний фактор є надзвичайно істотним у цьому контексті. Глутамат натрію - одна із найпоширеніших харчових добавок, яка часто застосовується неконтрольовано і може спричиняти зміни структури і функцій органів і тканин. Мета дослідження: проаналізувати динаміку морфологічних змін ділянки сонної пазухи під впливом глутамату натрію при пероральному введенні його в експерименті. Досліджено ділянку сонної пазухи 20 лабораторних білих щурів самців, що впродовж 8 тижнів отримували глутамат натрію перорально в дозі 10 мг/кг/добу, морфологічними методами на макро- та мікροструктурному рівнях через 6 і 8 тижнів експерименту. Отримані дані порівняно із результатами морфологічного дослідження цієї ж ділянки у 20 тварин контрольної групи. Статистичну обробку маси тварин проводили за допомогою програмного забезпечення MS Excel 2007 (визначали середню  $\pm$  середнє квадратичне відхилення). Через 6 тижнів експерименту при оцінці гістологічної будови стінки внутрішньої сонної артерії в зоні, що розташована безпосередньо над біфуркацією при порівнянні із контрольною групою в дослідній групі виявлено мультиплікацію і складчатість інтими, імовірно пов'язану із проліферацією клітин ендотелію під впливом глутамату натрію, відшарування ендотелію і лізис поодиноких ендотеліоцитів, а також нерівномірне потовщення еластичних волокон медії і порушення їх структури. Звертали на себе увагу накопичення білого жиру перивазально і в зоні сонного гломуса, а також дозороганізація нервів і розширення судин мікроциркуляторного русла. Через 8 тижнів експерименту була помітна негативна динаміка структурних змін: ознаки збільшення запальної інфільтрації, деформація судин мікроциркуляторного русла із потовщенням їх стінок і звуженням просвіту, стаз, помітна дегрануляція клітин сонного гломуса I типу, поява в інфільтраті поодиноких лаброцитів (мастоцитів). Помітно збільшилася також кількість жирової тканини (білий жир) в зоні сонної пазухи і біфуркації сонних артерій перивазально, а також в безпосередній близькості до каротидного гломуса, відмічалася тенденція до ущільнення жирової тканини. Таким чином, глутамат натрію при систематичному пероральному вживанні може спричиняти порушення структурної організації сонної пазухи, стінки сонних артерій і сонного гломуса, причому вираженість змін в динаміці наростає. Подальші дослідження необхідні для уточнення характеру структурних змін ділянки сонної пазухи за умов відміни глутамату натрію, а також пошуку можливих способів корекції.*

**Ключові слова:** глутамат натрію, сонна пазуха, сонний гломус, внутрішня сонна артерія, біфуркація сонних артерій.

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