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THE EFFECT OF MONOSODIUM GLUTAMATE CONSUMPTION ON CAROTID SINUS MORPHOLOGY: AN ELECTRON MICROSCOPY EXPERIMENTAL STUDY

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Monosodium glutamate (MG) is a popular food additive that is widely used for flavor enhancement. It is considered relatively safe for consumption in many countries since the time it was first discovered in 1907. However, various disorders have been attributed to MG exposure, while its toxic effects were reported in numerous studies. MG was associated with obesity, metabolic, gastrointestinal, reproductive and other disorders, while damage to satiety center was attributed to its use. However, some of the experimental studies conducted in the past had several flaws in their design or execution that presumably could have affected the results' extrapolation onto the human population. Further studies are needed to establish monosodium glutamate role in induction and progression of vascular pathology, particularly its effects on the morphology of carotid sinus and adjacent structures, as information on this particular issue is scarce. The objective of this study was to analyze early morphologic changes of carotid sinus under the influence of a low dose of monosodium glutamate administered orally by means of electron microscopy in experimental setting. Carotid sinuses of 10 adult male albino rats were studied by electron microscopy following 4, 6 and 8 weeks of oral administration of 10 mg/kg monosodium glutamate daily. The data was compared with the results of morphologic study of carotid sinus in the control group of 10 adult male albino rats. The data obtained suggests that monosodium glutamate oral consumption is associated with alterations of carotid sinus wall consistent with dystrophy and hypoxia at the early stages of exposure and apoptosis, fibrosis and lipid transformation at the later stages, while carotid glomus shows signs of cellular damage and apoptosis at a slightly later point in time but then the alterations worsen progressively. Further investigation is needed to evaluate morphologic changes of carotid sinus and adjacent structures associated with monosodium glutamate withdrawal.

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

Connection of the publication with planned research works.

This study is a fragment of planned scientific work of the Normal Anatomy Department and Topographic Anatomy and Operative Surgery Department «Morphologic and Functional Features of Organs in Pre- and Postnatal Periods of Ontogenesis under the Influence of Opioids, Food Additives, Reconstructive Surgeries and Obesity «, № of state registration 0120U002129.

Introduction.

Monosodium glutamate (MG), also known as monosodium salt of glutamic acid, or food additive E621, is widely used for taste enhancement of food that has partially lost some of its properties due to cooking or storing. MG was first discovered by Professor Kikunae Ikeda of Tokyo University in 1907. Since then, MG has been widely used in food industry thanks to its taste enhancing properties and relative safety. Food additive E621 is allowed for use in many countries and there is no unified approach when it comes to the safe dose of the afore-mentioned taste enhancer. MG use oftentimes is not strictly monitored: despite it being commonly added to many foods, some producers would not even mention its content on the packaging. First publication pertaining monosodium glutamate's possible role in the development of certain diseases had appeared in the British Medical Journal in 1968 [1]. This was the first time the term "Chinese restaurant syndrome" was applied. It was described as a combination of acute stomach pain, chest pain and headache, face flushing, fever and sweating that appeared after MG consumption [2, 3]. Unwavering interest of behalf of researches to MG has led to numerous studies being conducted in the recent decades that demonstrated an association between excessive MG consumption and inflammation, nervous system and gonadal damage [4], obesity and metabolic disorders [5, 6], atherosclerosis, hypertension, stroke and myocardial impairment [7], as well as cognitive disturbances [8]. However, some papers on the possible health risks associated with MG consumption published recently denied its negative effects or pointed towards them being variable or transient [9]. Given the importance of cardiovascular disorders in the mortality structure [10], the role of alimentary factors in risk modification and prevention of primary diseases and their relapses is hard to underestimate [11]. The underlying reason for cerebral circulation disturbances is often carotid arteries pathology [12], particularly atherosclerosis, and morphologic processes associated with its development have been studied recently with all possible means, including scanning electron microscopy that helped to establish peculiarities of mineral deposits' location in atherosclerotic plaques as well as to define the role of the damage caused by free radicals in the process of plaque formation [13, 14]. Carotid sinus morphology is of special interest in this context due to carotid glomus proximity and its direct involvement in the regulation of cerebral circulation given its structure and function [15] as well as ability to quickly react to hypoxia by activating nerve endings and setting off reflectory hyperventilation and sympathetic activation, notwithstanding its ability for hyperplasia in the setting of chronic hypoxia [16, 17]. At the same time, influence of potentially harmful factors, including MG, onto the morphology of carotid sinus and carotid glomus has not been fully studied and is of substantial interest given their prominent role in the regulation of cardiovascular function and cerebral circulation.

The aim of the study.

The aim of this study was to analyze morphologic changes of carotid sinus under the influence of a low dose of monosodium glutamate administered orally by means of electron microscopy in experimental setting.

Object and research methods.

20 male albino rats were enrolled into the study and equally divided into two groups: study and control ones. The rats were kept in cages, 4 animals in each one, and had unrestricted access to the standard vivarium food. The animals from the study group received 10mg/kg/d of MG orally for the duration of 8 weeks, while the control group received no food additives. The research was conducted in strict accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Council of Europe Directive 86/609/EEC (1986), the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Behavior" (2006), the General Ethical Principles on Animal Experimentation, approved by the First National Congress of Ukraine on Bioethics (2001).

The animals were withdrawn from the experiment using overdose of inhalation anesthesia at the end of weeks 4, 6 and 8; carotid arteries in the area of carotid bifurcation were harvested immediately. Ultrathin slices of the carotid sinus wall were prepared with ultramicrotome UZhTP-3 using glass knives. Slices of silver or lemon color were selected for the study. The specimens were first contrasted in 2% solution of uranyl acetate,

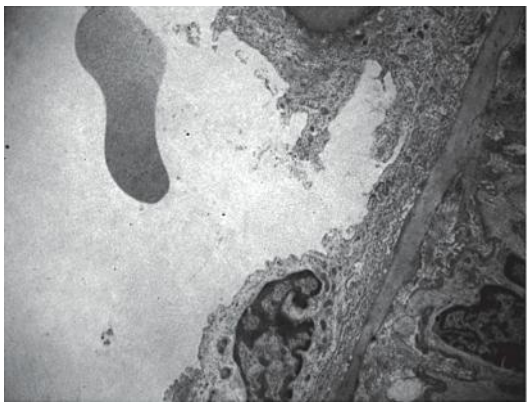

Figure 1 – A fragment of carotid sinus wall after 4 weeks of MG consumption. Microphotograph. Magnification: x1000.

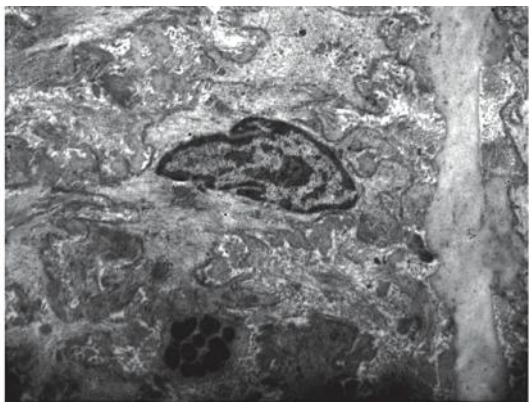

Figure 2 – A myocyte after 4 weeks of MG consumption. Microphotograph. Magnification: x4000.

Figure 3 – Collagen fibers after 4 weeks of MG consumption. Microphotograph. Magnification: x3000.

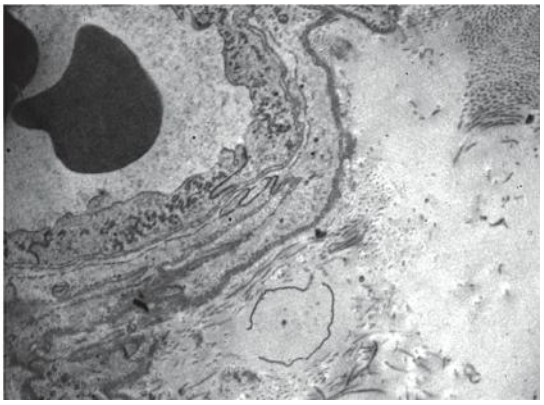

Figure 4 – Venule in the carotid sinus wall adventitia after 4 weeks of MG consumption. Microphotograph. Magnification: x4500.

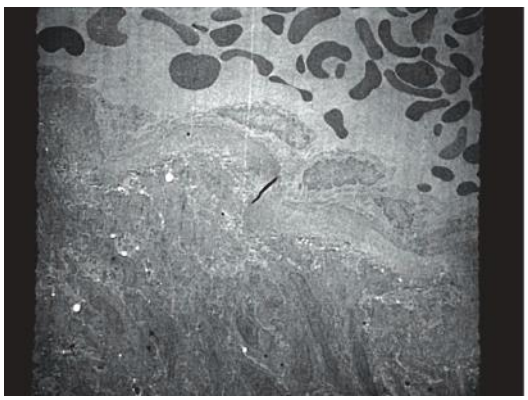

Figure 5 – A fragment of carotid sinus wall after 6 weeks of MG consumption. Erythrocytes in the lumen. Microphotograph. Magnification: x1000.

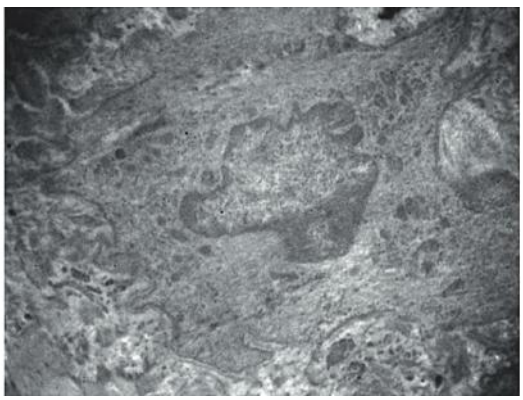

Figure 6 – Myocyte after 6 weeks of MG consumption. Microphotograph. Magnification: x6000.

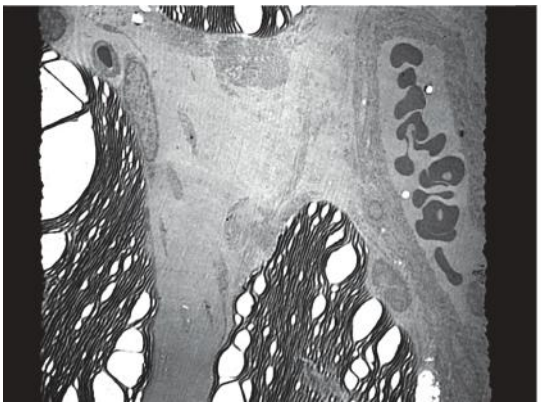

Figure 7 – Collagen fibers after 6 weeks of MG consumption. Microphotograph. Magnification: x1000.

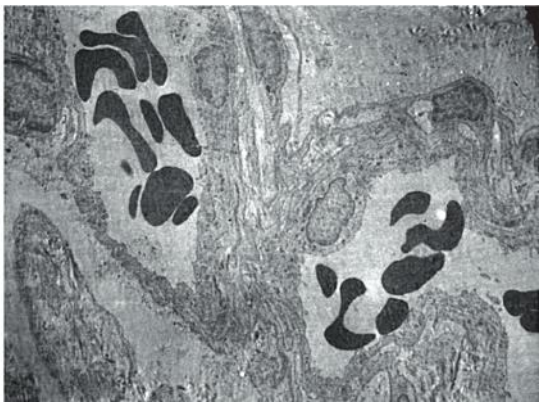

Figure 8 – Venules in the carotid sinus adventitia after 6 weeks of MG consumption. Microphotograph. Magnification: x1500.

Figure 9 – A fragment of the internal carotid artery wall after 8 weeks of MG consumption. Microphotograph. Magnification: x2000.

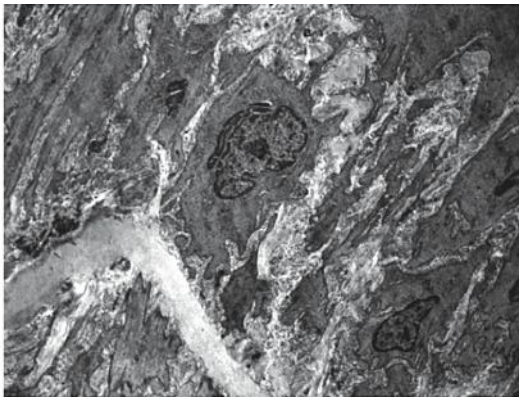

Figure 10 – Myocyte after 8 weeks of MG consumption. Microphotograph. Magnification: x2000.

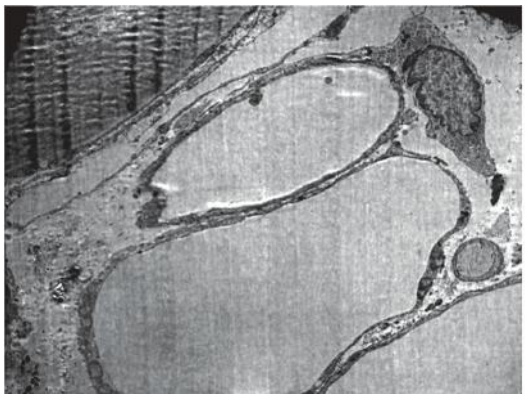

Figure 11 – Adventitia of the carotid sinus after 8 weeks of MG consumption. Microphotograph. Magnification: x1500.

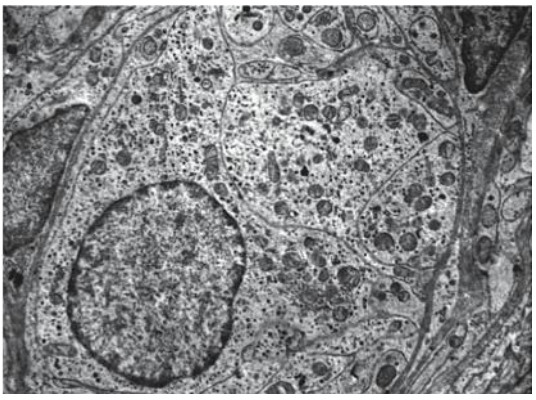

Figure 12 – Carotid glomus after 4 weeks of MG consumption. Microphotograph. Magnification: x4000.

and then in lead citrate. The material was studied and photographed using electron microscope УЕМВ-100 К with acceleration voltage of 75 kV and magnification of x1000-x7500.

Research results and their discussion.

Upon assessment of the experimental material, at 4 weeks from the start of oral MG consumption in the dose of 10 mg/kg/d, electron microscopy revealed changes in the endothelium of the carotid sinus wall, probably associated with hypoxia: cytoplasmic membrane protrusions, known also as microvilli **(fig. 1)**, moderate deformity of the cytoplasmic membrane of the smooth myocytes of the media, partial destruction of karyolemma of some of their nuclei with chromatin condensation within them **(fig. 2)**. As for the collagen fibers, it was noted that some of their fibrils were unevenly thickened, occasionally dissected (**fig. 3)**. Adventitia of the carotid sinus wall was edematous with vasa vasorum clearly seen within it **(fig. 4)**.

6 weeks from the start of the experiment, negative dynamics of the changes was observed: endotheliocytes showed dystrophic changes, vacuoles, signs of peeling off, dissection and microvilli **(fig. 5)**, while myocytes had deformed contours, lipid inclusions, lateral position of chromatin, invaginations of karyolemma, nuclei fragmentation that were characteristic of apoptosis, as well as damaged intercellular contacts **(fig. 6)**. As for adventitia, there was white fat observed in-between the collagen fibers **(fig. 7)**, while in the deformed venular lumen

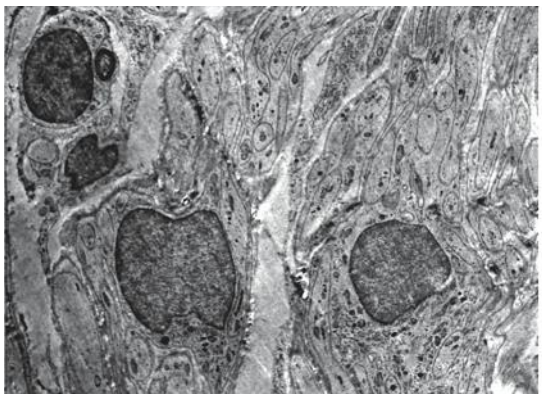

Figure 13 – Carotid glomus after 6 weeks of MG consumption. Microphotograph. Magnification: x2000.

Figure 14 – Carotid glomus after 8 weeks of MG consumption. Microphotograph. Magnification: x4000.

distorted erythrocytes were seen, pointing towards hypoxia **(fig. 8)**.

8 weeks into the experiment the internal layer of the carotid sinus wall was remarkable for pathologically changed endotheliocytes with fragmented heterogeneous nuclei, at times too lucent or, on the contrary, too electron-dense, pronounced protrusions of the cytoplasmic membrane and deformity of the basal membrane, as well as heterogeneous dilation of the endoplasmic reticulum channels, both in smooth and granular reticula **(fig. 9)**. Assessment of media resulted in visualization of dissected collagen fibers, karyopyknosis and apoptosis of the myocytes **(fig. 10).** While the adventitia was remarkable for the presence of considerable amounts of fat, giant lipocytes and fibroblasts – all signs of sclerosis and fatty transformation **(fig. 11)**.

As for the carotid glomus, 4 weeks into the experiment it didn't differ from the control significantly, had typical structure and was comprised of two types of cells: glomus and sustentacular ones, with nuclei of regular round shape and normal chromatin positioning **(fig. 12)**. 6 weeks into the experiment the glomus cells nuclei had changed their shape, showed heterogenic density, their karyoplasm was mostly filled with condensed chromatin, in some areas lateral chromatin positioning was noted **(fig. 13)**. At week 8 of the experiment, considerable negative dynamics was observed in the structural organization of the nuclei that showed deep invaginations of karyolemma, karyopyknosis and karyolysis being the signs of apoptosis of the glomus cells **(fig. 14)**.

Conclusions.

The data obtained points towards a connection between systematic low dose MG consumption and pathological changes of structural organization of the carotid sinus wall and the carotid glomus that are seen even at an early stage and tend to progress with time manifesting in epithelium dystrophy, apoptosis of the smooth muscle cells of the media and glomus cells, as well as fibrosis and fat infiltration of the adventitia of the vascular wall. Pathologic changes in the carotid glomus, namely apoptosis of the cells, are noted at a later time but show a tendency towards rapid progression. Understanding the structural changes of the carotid sinus and adjacent structures under the influence of MG, their degree and potential for reversal can facilitate prophylaxis and treatment of cerebral circulation disorders, as well as to serve as a background for risk factors modulation to make prevention of these disorders more efficient and successful.

Perspectives for further research.

Further research is required to establish the character, degree and nature of morphologic changes of carotid sinus, carotid arteries walls and carotid glomus in the setting of monosodium glutamate withdrawal. Besides, searching for agents with a possible corrective potential in the setting of chronic MG exposure has considerable practical sense and a potential for clinical implementation.

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УЛЬТРАСТРУКТУРНІ ЗМІНИ ДІЛЯНКИ СОННОЇ ПАЗУХИ ЗА УМОВ ВПЛИВУ ГЛУТАМАТУ НАТРІЮ В ЕКСПЕРИМЕНТІ

Содомора О. О.

Резюме. *Вступ.* Глутамат натрію (Е621) – одна з найпоширеніших харчових добавок, яка вважається відносно безпечною і широко застосовується в харчовій промисловості. Однак віддавна існують застереження щодо можливого негативного впливу добавки Е621 на живий організм, особливо при тривалому пероральному застосуванні. В численних наукових дослідженнях виявляли токсичні ефекти глутамату натрію на органи і тканини, однак з огляду на різноманітний дизайн досліджень, застосування доз, які не завжди відповідали загальноприйнятим в харчовій промисловості, а також відмінні від перорального шляхи введення глутамату, практичне застосування результатів окремих наукових робіт було дещо обмеженим. Нечисленні і подекуди суперечливі відомості щодо впливу невисоких доз глутамату натрію на ультраструктурну організацію судин, зокрема сонної пазухи, стали передумовою цього дослідження.

Мета – проаналізувати ультраструктурні зміни сонної пазухи під впливом глутамату натрію при пероральному введенні його в експерименті в дозах, що вважаються безпечними.

Об'єкт і методи дослідження. Досліджено ділянку сонної пазухи 10 лабораторних білих щурів самців, що впродовж 8 тижнів отримували глутамат натрію перорально в дозі 10 мг/кг/добу, методом електронної мікроскопії. Отримані дані порівняно із результатами морфологічного дослідження цієї ж ділянки у 10 тварин контрольної групи.

Висновки. Отримані дані свідчать про те, що систематичне вживання в їжу глутамату натрію навіть в невеликих дозах вже на ранніх термінах може бути пов'язане із патологічними змінами структурної організації стінки сонної пазухи і порушеннями морфології сонного гломуса, які в динаміці наростають і характеризуються дистрофічними змінами ендотелію, апоптозом клітин медії та сонного гломуса, фіброзом і жировою інфільтрацією адвентиції судинної стінки. Патологічні зміни клітин сонного гломуса, що мають характер апоптозу, починаються дещо пізніше, але швидко прогресують в динаміці. Розуміння особливостей, ступеня вираженості і оборотності структурних змін ділянки сонної пазухи під впливом глутамату натрію може сприяти лікуванню і профілактиці порушень мозкового кровообігу, а також створити підгрунтя для моделювання потенційних факторів ризику розвитку цих патологічних процесів.

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

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Sodomora O. O.

Abstract. *Background.* Monosodium glutamate is a food additive and flavor enhancer that is widely used and considered relatively safe for consumption in many countries. Since it was first discovered by Professor Kikunae Ikeda in 1907, its various toxic effects were reported in numerous studies. However, not all of them were equally decisive in their conclusions given the fact some of the experimental studies conducted had several flaws in their design that presumably could have affected the results' extrapolation onto the human population. Further studies are needed to establish monosodium glutamate role in vascular pathology induction and development, particularly its effects on the morphology of carotid sinus and adjacent structures.

Objective. The objective of this study was to analyze morphologic changes of carotid sinus under the influence of a low dose of monosodium glutamate administered orally by means of electron microscopy in experimental setting.

Materials and methods. Carotid sinuses of 10 adult male white laboratory rats were studied by electron microscopy following 4, 6 and 8 weeks of oral administration of 10 mg/kg monosodium glutamate daily. The data was compared with the results of morphologic study of carotid sinus in the control group of 10 adult male rats.

Conclusions. The data obtained suggests that monosodium glutamate oral consumption is associated with alterations of carotid sinus wall consistent with dystrophy and hypoxia at the early stages and apoptosis, fibrosis and lipid transformation at the later stages, while carotid glomus shows signs of cellular damage and apoptosis at a slightly later point in time but then the alterations worsen progressively. Further investigation is needed to evaluate morphologic changes of carotid sinus and adjacent structures associated with monosodium glutamate withdrawal.

Key words: monosodium glutamate, carotid sinus, carotid glomus, internal carotid artery, carotid bifurcation.

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A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article.

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