

G.A. Yeroshenko, Yu.V. Tymoshenko
 HSEE of Ukraine "Ukrainian Medical Stomatological Academy", Poltava

THE DYNAMICS OF EXPRESSION OF THE CARBOHYDRATE DETERMINANTS OF STRUCTURAL COMPONENTS OF MUCOUS MEMBRANE OF GLANDULAR ZONE OF HARD PALATE IN EXPERIMENTAL HYPOSALIVATION

e-mail: gala_umsa@ukr.net

The sounding of the hard palate mucosa by β -Gal specific peanut lectin (PNA) in the experimental hyposalivation has shown the suppression of marking intensity of the epithelial lamina layers during observation. The resident components of the connective tissue of the lamina propria (fibroblasts and collagen fibers) demonstrated the increase in the intensity of reactions from weak to moderate one. The persistent high-degree conjugation with galactose-specific receptors of mastocytes throughout the entire period of observation was noteworthy. The lowering of the binding specificity up to weak intensity of carbohydrate determinants' exposure to peanut lectin on the basal membrane and plasmolemma (strong in the intact group, very strong on the 14th day of the observation) was established in the acini. The expression of myoepithelial receptors reduced from very strong to weak in the intact group. The study of the specificity with the receptors on the structural components of the basal membrane and basal plasmolemma of the excretory ducts of submandibular glands has revealed a weak reaction in the intact rats, which was increasing to strong one on the 14th day of the experiment, though it demonstrated a weak reaction on day 30 of the observation.

Keywords: mucous membrane, hard palate, rats, hyposalivation.

The paper has been written within the RSW "Experimental and morphological study of the effect of cryopreserved placenta transplants and other exogenous factors on morphofunctional state of some internal organs", State registration number 0113U006185.

Minor salivary glands are involved in the formation of the oral fluid components. Moreover, they support antioxidation activity and contribute to the formation of the local immunity. The impact of different factors can lead to reduction of secretory activity of salivary glands, i.e., hyposalivation, which is clinically manifested by xerostomia. The carbohydrate structures of varying complexity on the surface of cells are ligands for binding with lectins, thereby affecting the processes of functioning of the cells, tissues and organs [1, 4]. Basically, lectins are involved into cell recognition, for example, some pathogens use lectins to be attached to the cells of the affected body. Lectins possess a selective activity with respect to multiple subpopulations of the cells [3, 9]. The method of lectin sounding with its sensitivity and selectivity to detection of the specified molecular structures has more advantages over the traditional methods of histochemical detection of carbohydrate determinants [8].

The purpose of the study was to determine the dynamics of the expression of carbohydrate determinants of the structural components of the mucous membrane of the glandular zone of the hard palate for β -Gal specific peanut lectin in experimental hyposalivation.

Material and Methods. 30 white outbred male rats were involved into the study. 5 animals were assigned into control group and 10 animals were assigned into the experimental group. Xerostomia was induced by washing of rat oral mucosa with 1% methacrylic acid methyl ether solution during 30 days [2]. The animals were killed on day 14 and 30 of the experiment under thiopental anesthesia overdose.

The carbohydrate residues of galactose were detected by the peanut lectins (PNA). The specimens were treated with the standard sets of "Lectinotest" laboratory (Lviv) in 1:50 lectin dilution [5].

Visualization of the reaction with lectin conjugates was performed by the semiquantitative method in the Biorex - 3 VM – 500 microscope immersion magnifications [10].

Animal housing and experiments on them have been carried out in compliance with the "General Ethic Rules for Conducting Experiments on Animals", adopted by the I National Congress on Bioethics [6] and the requirements of international principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [7].

Results and Discussion. The sounding of the hard palate mucosa in the intact rats by β -Gal specific peanut lectin (PNA) demonstrated a strong degree of the conjugation with receptors of horny scales. The reaction of the cells of stratum granulosum and stratum spinosum, as well as basal membrane was moderate. The cells of the stratum basale showed weak reaction. The intensity of marking of the components of the lamina propria was weak, the lymphocytes showed moderate expression and strong reaction was detected in mastocytes (Fig. 1). The reaction of the components of the elastic membrane to β -Galactose was negative. The affinity of the structural components of the palatine glands of the intact rats to β -Galactose was strong in the epithelial cells of the acini, namely, the basal membrane and basal plasmalemma. The intensity of marking of the surface and cytoplasm of the myoepitheliocytes was very strong (Fig.2). Myoepitheliocytes of the

excretory ducts showed strong affinity of β -Galactose. The expression of receptors of the basal membrane, basal plasmolemma and cytoplasm of the ductal epithelial cells was weak.

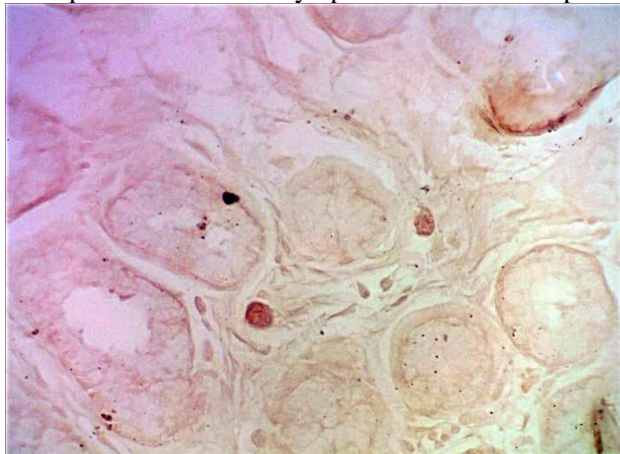


Fig. 1. Strong expression of the β -Gal specific peanut lectin on the mastocytes in the stroma of the salivary glands of the glandular zone of hard palate of intact rat. PNA marking. Magnification: Lens $\times 100$. Ocular lens $\times 10$.

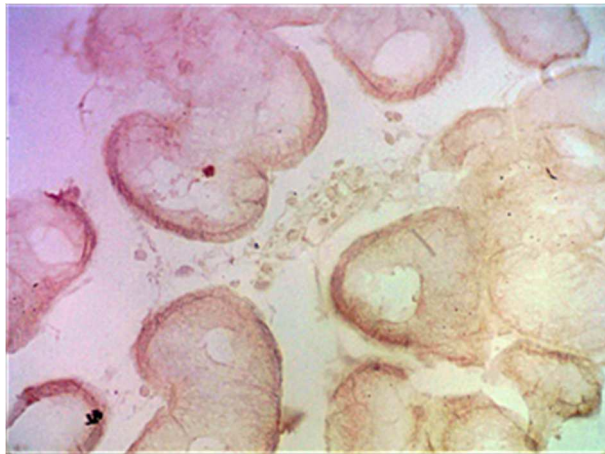


Fig. 2. Strong expression of the β -Gal specific peanut lectin on the plasmolemma of the secretory cells of the acini and myoepitheliocytes of the salivary glands of the glandular zone of hard palate of intact rat. PNA marking. Magnification: Lens $\times 100$. Ocular lens $\times 10$.

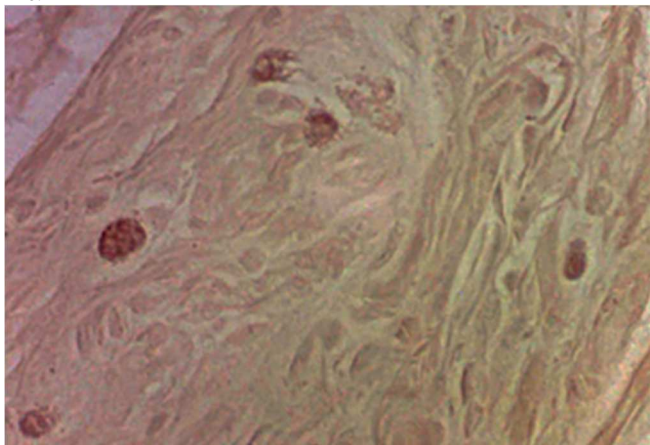


Fig.3. Strong expression of the β -Gal specific peanut lectin on the collagen fibers and mastocytes in the lamina propria of the glandular zone of hard palate on day 14 of the experiment. PNA marking. Magnification: Lens $\times 100$. Ocular lens $\times 10$.

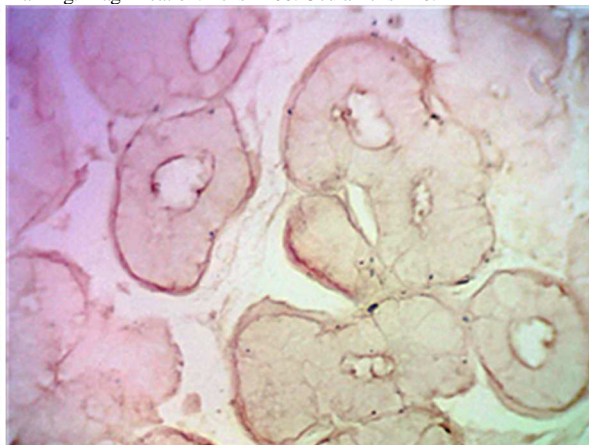


Fig. 4. Very strong expression of the β -Gal specific peanut lectin on the basal parts of the acinar epithelial cells of the salivary glands in the glandular zone of rat hard palate on the 30th day of the experiment. PNA marking. Magnification: Lens $\times 100$. Ocular lens $\times 10$.

On the 14th day of the observation the intensity of binding of the receptors of horny scales with peanut lectin remained unchanged, strong. The expression of the cells of the stratum granulosum increased. The intensity of marking of the cells of stratum spinosum declined to the weak one. The affinity of the basal membrane remained unchanged. The study of the specificity of binding of the components of lamina propria has determined the enhancement of the expression of receptors to β -Galactose by fibroblasts. The reaction of the collagen fibers, components of the vascular wall (weak) and mastocytes (strong) remained at the level of the intact rats (Fig. 3). The exposure of the peanut lectin receptors on lymphocytes declined from moderate to weak one. Macrophages, on the contrary, increased the expression of receptors from null to strong one. The degree of exposure of the peanut lectin receptors on lymphocytes declined from moderate to low one. Macrophages, on the contrary, increased the expression of receptors from null to strong one. The enhancement of the expression to the peanut lectin on the basal membrane and basal plasmolemma of the acini of rat submandibular glands from strong to very strong was established on the 14th day of the experiment. The reduction of the receptors to β -Galactose by the myoepitheliocytes of the acini and excretory ducts from very strong to moderate one was detected. The horny scales showed the declined reaction (from strong to moderate one) in the epithelial lamina of the glandular zone of the rat hard palate mucosa on the 30th day of the observation. Keratinocytes of the stratum granulosum, stratum spinosum, stratum basale and basal membrane showed weak intensity of the reaction. In the lamina propria the expression of the receptors to peanut lectin remained moderate (weak in the intact animals) on the cytoplasm of the fibroblasts. The degree of binding by collagen fibers and vascular endothelial cells enhanced from weak to moderate one. The reaction of the basal membrane of the vessels and elastic membrane of arterioles was unchanged. Among the migrant cells of connective tissue the mastocytes showed a strong expression of the receptors, whilst lymphocytes and macrophages showed no reaction. The acini of the submundibular glands showed a declined intensity (up to

weak one) of the carbohydrate determinants exposure to the peanut lectin on the basal membrane and plasmolemma (strong in the intact group and very strong on day 14 of the observation) on the 30th day of the experiment. The expression of the receptors to the PNA lectin declined from the moderate to weak one on the myoepitheliocytes (very strong in the intact group and moderate on the day 14 of the experiment) (Fig.4).

On the 30th day of the observation the study of the specific binding of β -Gal specific peanut lectin with the receptors of structural components of the excretory ducts of submandibular glands showed weak reaction of the basal membrane and basal plasmolemma (weak in the intact rats and strong on the 14th day of the experiment), in the cytoplasm and myoepitheliocytes (very strong in the intact animals and weak on the 14th day of the experiment).

Conclusion

The sounding of the hard palate mucosa by β -Gal specific peanut lectin (PNA) in the experimental hyposalivation has shown the suppression of marking intensity of the epithelial lamina layers during observation. The resident components of the connective tissue of the lamina propria (fibroblasts and collagen fibers) demonstrated the increase in the intensity of reactions from weak to moderate one. The persistent high-degree conjugation with galactose-specific receptors of mastocytes throughout the entire period of observation was noteworthy. The lowering of the binding specificity up to weak intensity of carbohydrate determinants' exposure to peanut lectin on the basal membrane and plasmolemma (strong in the intact group, very strong on the 14th day of the observation) was established in the acini. The expression of myoepithelial receptors reduced from very strong to weak one in the intact group. The study of the specificity with the receptors on the structural components of the basal membrane and basal plasmolemma of the excretory ducts of submandibular glands has revealed a weak reaction in the intact rats, which was increasing to strong one on the 14th day of the experiment, though it demonstrated a weak reaction on day 30 of the observation.

References

1. Antoniuk RV., Lutsyk OD. Lektynohistokhimichne doslidzhennia tovstoy kysky liudyny v normi ta pry neoplastychnykh protsesakh z vykorystanniam lektyniv, spetsyfychnykh do T-antychenu ta N-atsetyllaktozaminu. Svit medytsyny ta biolohii. 2015; 2(49): 74-80.
2. Babii R.I. Korektsiia funktsionalnoi aktyvnosti slynykh zaloz pry zubnomu protezuvanni khvorykh z hiposalivatsiieiu: dys. na zdbuttia nauk. stupenia kand. med. nauk:14.01.22 «stomatolohiia». Odessa. 2008: 143.
3. Bilash SM, Yeroshenko GA, Pokotylo PB. Lektynokhimichna kharakterystyka vuhlevodnykh determinant shlunku pry vvedenni kriokonservovanoi platsenty na tli hostroho eksperymentalnoho zapalennia. Svit medytsyny ta biolohii. 2013; 1 (36): 94 – 99.
4. Vasylichuk NH, Kushch OH. Osoblyvosti rozpodilu retseptoriv do lektyniv arakhisu ta ikry okunia v kapsuli mediastenalnoho limfatychnoho vuzla. Svit medytsyny ta biolohii. 2015; 4(53): 15-18.
5. Lutsik AD, Detyuk ES, Lutsik MD. Lektyny v gistohimii. Lvov: Vischa shkola. 1989: 140.
6. Obschieeticheskie printsypy raboty s eksperymentalnyimi zhivotnyimi pri provedenii meditsynskikh i biologicheskikh issledovaniy / Natsionalniy kongres z bioetiki (Kiyiv 17 – 20 veresnya 2001 r.). Zh. AMN Ukrainy. 2001; 7, (4): 814 – 816.
7. European convention for the protection of vertebrate animals used for experimental and other scientific purposes. - Strasbourg: Council of Europe. 1986: 53.
8. Katsuyama T, Spicer S. Histochemical differentiation of complex carbohydrates with variants of the concanavalin A-horseradishperoxidase method. J. Histochem. Cytochem. 2008; 26 (4): 233-250.
9. Villalobo A, Nogales-Conzalez A, Galius H. A guide to signaling pathways connecting protein-glycan interaction with the emerging versatile effector functionality of mammalian lectins. Trends Glycosci. Glycotechnol. 2006; 18: 1–37.
10. Yeroshenko G, Timoshenko Yu, Kazakova K, Yeroshenko A. Lectinochemical characteristics of rat normal masticatory oral mucosa The XV International Academic Congress «Fundamental and Applied Studies in the Modern World». United Kingdom, Oxford. 2016; XV: 207 – 211.

Реферати

**ДИНАМІКА ЕКСПРЕСІЇ ВУГЛЕВОДНИХ
ДЕТЕРМІНАНТ СТРУКТУРНИХ КОМПОНЕНТІВ
СЛИЗОВОЇ ОБОЛОНКИ ЗАЛОЗИСТОЇ ЗОНИ
ТВЕРДОГО ПІДНЕБІННЯ ЗА УМОВ
ЕКСПЕРЕМЕНТАЛЬНОЇ ГИПОСАЛІВАЦІЇ**
Єрошенко Г.А., Тимошенко Ю.В.

Зондування слизової оболонки твердого піднебіння β -галактозоспецифічним лектином арахісу (PNA) при експериментальній гіпосалівації визначило пригнічення інтенсивності маркування шарів епітеліальної пластинки протягом спостереження. Резидентні компоненти сполучної тканини власної пластинки (фібробласти і колагенові волокна) показали збільшення інтенсивності реакції від слабкої до помірної. Необхідно зазначити стабільно сильний ступень кон'югації з галактозоспецифічними рецепторами мастоцитів протягом всього терміну спостереження. З боку кінцевих відділів встановлено зниження специфічності

**ДИНАМИКА ЭКСПРЕССИИ УГЛЕВОДНЫХ
ДЕТЕРМИНАНТ СТРУКТУРНЫХ КОМПОНЕНТОВ
СЛИЗИСТОЙ ОБОЛОЧКИ ЖЕЛЕЗИСТОЙ ЗОНЫ
ТВЕРДОГО НЕБА В УСЛОВИЯХ
ЭКСПЕРИМЕНТАЛЬНОЙ ГИПОСАЛИВАЦИИ**
Ерошенко Г.А., Тимошенко Ю.В.

Зондирование слизистой оболочки твердого неба β -галактозоспецифическим лектином арахиса (PNA) при экспериментальной гипосаливации определило угнетение интенсивности маркировки слоев эпителиальной пластинки в течение эксперимента. Резидентные компоненты соединительной ткани собственной пластинки (фибробласты и коллагеновые волокна) показали увеличение интенсивности реакции от слабой до умеренной. Необходимо отметить стабильно сильную степень конъюгации с галактозоспецифическим рецепторами тучных клеток в течение всего срока наблюдения. Со стороны концевых отделов выявлено снижение специфи-

зв'язування до слабкої інтенсивності експонування вуглеводних детермінант до лектину арахісу на базальній мембрані та плазмолемі (сильна в інтактній групі, дуже сильна на 14 добу спостереження). З дуже сильної в інтактній групі, до слабкої зменшилась експресія рецепторів міоєпітеліоцитів. Дослідження специфічності з рецепторами структурних компонентів базальної мембрани і базальної плазмолемі вивідних проток піднебінних залоз виявило слабку реакцію у щурів інтактної групи, яка посилюється до сильної на 14 добу експерименту, але на 30 добу спостереження дає слабку реакцію.

Ключові слова: слизова оболонка, тверде піднебіння, щури, гіпосаливації.

Стаття надійшла 20.11.2017 р.

ності связывания до слабой интенсивности экспонирования углеводных детерминант к лектину арахиса на базальной мембране и плазмолемме (сильная в интактной группе, очень сильная на 14 сутки наблюдения). С очень сильной в интактной группе к слабой уменьшилась экспрессия рецепторов миоэпителиоцитов. Исследование специфичности с рецепторами структурных компонентов базальной мембраны и базальной плазмолеммы выводных протоков небных желез выявило слабую реакцию у крыс интактной группы, которая усилилась до сильной на 14 сутки эксперимента, но на 30 сутки наблюдения давала слабую реакцию.

Ключевые слова: слизистая оболочка, твердое небо, крысы, гипосаливация.

Рецензент Білаш С.М.

DOI 10.26.724 / 2079-8334-2018-1-63-126-130

UDC 616.12 + 616.379-008.64

Zhurakivska O.Ya., Mykulets T.L., Dutchak U.M., Klypych Ya.I., Miskiv V.A., Hrechyn A.B., Klypych O.O.
Ivano-Frankivsk National Medical University, Ivano-Frankivsk

STRUCTURAL CHANGES OF ENDOCRINE SYSTEM OF MYOCARDIUM DURING THE STREPTOZOTOCIN DIABETES MELLITUS

e-mail: zhurakivska.o.ya@gmail.com

The aim of the work was to establish the peculiarities of structural rearrangement of secretory atrial cardiomyocytes in the early and long-term streptozotocin diabetes mellitus (DM). DM was modeled by single intraperitoneal administration of streptozotocin (6 mg per 100 g of body weight). The material for the study was taken on the 14th and 56th days of the experiment. An electron microscope method of investigation was used. It was found that streptozotocin DM in secretory atrial cardiomyocytes leads to rearrangement of intracellular organelles responsible for the synthesis and secretion of atrial natriuretic peptide. It should be noted that there is a redistribution of various types of secretory granules (SG) in response to hyperglycemia. On the 14th day of experiment, there was a significant increase of the bulk density of diffusing SG, indicating the enhancement of withdrawal processes of ANP from the cell. On the 56th day, the bulk density of young and mature SG was significantly reduced, indicating a breakdown of compensatory mechanisms.

Key words: streptozotocin diabetes mellitus, rats, secretory atrial cardiomyocyte.

The article uses RSW "Optimization of complex treatment of morphological lesions of digestive, endocrine and urogenital systems in diabetes mellitus" (State registration number 0113U000769).

Over the past decades, scientists around the world have been studying the endocrine function of the heart [2, 15 and 22]. Since 1981, thanks to research by A.J. De Bold and co-authors who found that a substance with expressed natriuretic and diuretic properties was produced in the atria, the heart began to be considered as an organ that, along with the pump function, also carries out endocrine [20]. In 1983, a polypeptide, called ANP was isolated and purified from the heart of a rat. In the same year, this polypeptide was isolated from human atria [8]. The mechanical expansion of atria of the myocardium increases the secretion of ANP. The role of glucagon-like peptide-1 (GLP-1) in the regulation of ANP secretion by atria is also proved. GLP-1 is an incretin-like hormone secreted by endocrine cells of the epithelium of the small intestine. Stimulated by glucose, with the bloodstream, GLP-1 enters the B-cells of pancreas, which stimulates the release of insulin. The activation of the GLP-1 receptor found in the atria contributed to the release of ANP and the reduction of arterial pressure [10]. In modern scientific literature there are a lot of facts about endocrine effects of ANP, particularly its participation in the regulation of homeostasis [17, 18]. This peptide is an antagonist of the renin-angiotensin-aldosterone system, which is activated in DM. The morphological aspects of the condition of the endocrine heart apparatus in diabetes are insufficiently studied.

The purpose of paper was to establish the peculiarities of structural rearrangement of secretory atrial cardiomyocytes on the 14th and 56th days of streptozotocin DM.

Materials and methods. Pieces of atria of 20 white male rats of the Wistar line were used as a material for the study. Animals were divided into 2 groups (10 animals in each group): control and experimental. Experimental diabetes mellitus (EDM) in animals of the experimental group (EG) was modeled by a single intraperitoneal injection of streptozotocin (dissolved in 0.1M citrate buffer solution with pH 4.5) at a dose of 6mg per 100g of body weight. The control group (CG) of animals in an equivalent dose was injected intraperitoneally with 0.1M citrate buffer with pH 4.5. Euthanization of animals was performed under thiopental anesthesia by decapitation and subsequent collection of blood in a test tube for biochemical studies.