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 міоепітеліоцитів. Дослідження специфічності з рецепторами структурних компонентів базальної мембрани і базальної плазмолеми 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, гіпосалівації.

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чности 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 миоэпителиоцитив. 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.

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

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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 14^{th} 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 56^{th} 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 where 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.

The development of diabetes was controlled by the daily determination of blood glucose level, which was measured with a drop of blood of the caudal vein using test strips at the AccuCheck (Germany) glucose meter. The level of glycated hemoglobin (HbA1c) in blood was determined in the "Diameb" certified laboratory using the ACCENT-200 HbA1c DIRECT diagnostic kit (PZ Cormay S.A., Poland). The materials were taken on the 14th and 56th days of the experiment. An electron microscopic method of investigation was used. The bulk density of SGs were determined.

Morphometry was performed on digitized electronograms using the ImageJ v software. 1.47 (NIH, USA, http://imagej.nih.gov/ij) NIH USA "Image J" in manual mode with allowance for increments. Computer data processing was carried out using the statistical package Stat.Soft.Inc; Tulsa, OK, USA; Statistica 6.

Results and their discussion. In the control group of animals, with the use of the electron microscope, we, like other authors [5], distinguished three types of SG: Type 1 (young) were characterized by a matrix of high electron density, which was surrounded by a membrane with a light rim beneath; Type 2 (mature) had a matrix less electronically tight, there was a membrane, but the sub-membrane rim was absent; Type 3 (diffusing) had a matrix of moderate density, with no outer membrane, and the periphery was blurred. Sometimes the contours of diffusing SGs were blurred, some of them became crescent shaped, indicating the secretion from them to the bloodstream. A low electro-optical density of the 3rd type of SG is associated with the release of ANP out of them by exocytosis, which led to a decrease in their optical density [19, 6 and 13]. In the control group of animals, young and mature SGs were located mainly near the nucleus and under the sarcolemma in the diffusing type. Although, all three types of SGs were detected in the area of the Golgi complex, around the nucleus. On the 14^{th} day of the experimental DM, the blood glucose level was 16.78 ± 0.14 mmol / l (control 5.09 ± 0.25 mmol / l, $p < 0.001$), HbA1c 6.54 ± 0.75% (control - 1.88 ± 0.31%, $p < 0.01$). At the ultra-structural level, the clarification of the sarcoplasm of secretory atrial cardiomyocytes of different parts of the heart was revealed. Sarcolemma was loose and sometimes formed invaginations inside the cell. In some secretory atrial cardiomyocytes, hypertrophy and hyperplasia of structural components of the Golgi complex, granular endoplasmic mesh, were found. The nuclei of secretory atrial cardiomyocytes contained diffusely located granules of euchromatin (Fig. 1a).

Nuclear shell formed numerous non-deep finger-like protrusions. Near the nucleus, there were single young and mature SGs, most of them, along with diffusing SGs, were located sub-sarcolemmally (Fig. 1b). According to the morphometric analysis (Table 1), the volume density of SGs at this time of experiment was significantly increased due to all their forms, especially young ones. It should be noted that the volume density of diffusing SGs increased by almost 40%, which obviously lead to an increase of ANP in blood. One of the main factors contributing to the release of ANP from the atria is mechanical stretching of the myocardium [11]. From the pathogenetic point of view, the increase of the polyol route of glucose metabolism in DM increases the formation and accumulation of sorbitol in tissues, which is accompanied by an increase in osmolality and the amount of intracellular fluid with tissue damage. Some authors are convinced that moderate hyperglycemia and chronic increase in plasma volume stimulate the release of ANP [1], which is confirmed by our research data. ANP, in turn, leads to an increase in the velocity of glomerular filtration and polyuria [16]. Some researchers on the 17th day of the development of experimental streptozotocin diabetes observed a significant increase in the level of ANP in rat's blood. They considered that pathogenetic mechanism of development of this phenomenon was the decrease in the sensitivity of kidney receptors to ANP. This was confirmed by clinical observations. Thus, in patients with type 2 DM with the presence of microalbuminuria in the background of diabetic nephropathy, more than 27% increase in the level of natriuretic peptides in blood plasma was observed [23]. Other authors believe that an ischemia and interstitial edema [3], which we observed in the myocardium in the long-term course of diabetes mellitus, was also a stimulant for accumulation and enhanced allocation of ANP. In turn, endothelin, as a strong vasoconstrictor, also increases the secretion of ANP [4, 7, 12]. The most pronounced changes during the period of observation were found in mitochondria. Most of them had the illuminated matrix, part of them had discomplexation of the cristae, and in the third ones we observed the complete destruction of the inner shell with the formation of vacuoles. On the 56^{th} day of experimental DM, glucose and HbA1c levels increased to 18.21 ± 0.22 mmol / L (control 5.31 ± 0.23 mmol / l, $p < 0.001$) and 9.31 ± 0.25% (control - 2,32 ± 0,09%, $p < 0,01$), indicating the development of a severe decompensated form of diabetes. In secretory atrial cardiomyocytes we observed the phenomena of vacuolar and hydroponic dystrophy (Fig. 2a). Structural elements of the Golgi complex were destroyed, granular endoplasmic cisternae were expanded with single ribosomes attached to them. Most mitochondria were swollen, had an enlightened matrix and destroyed cristae. Quite often we observed edema in sub-sarcolemmal region and around the nucleus, lysis of myofibrils and their rupture (Fig. 2b). The bulk density of SGs were decreased, compared to the previous study period, due to their young and mature forms (see Table 1). At the same time, the bulk density of diffusing SGs did not significantly change and was greater

than the control values, this also applied to the bulk density of all SGs. Most of the SGs were located under sarcolemma.

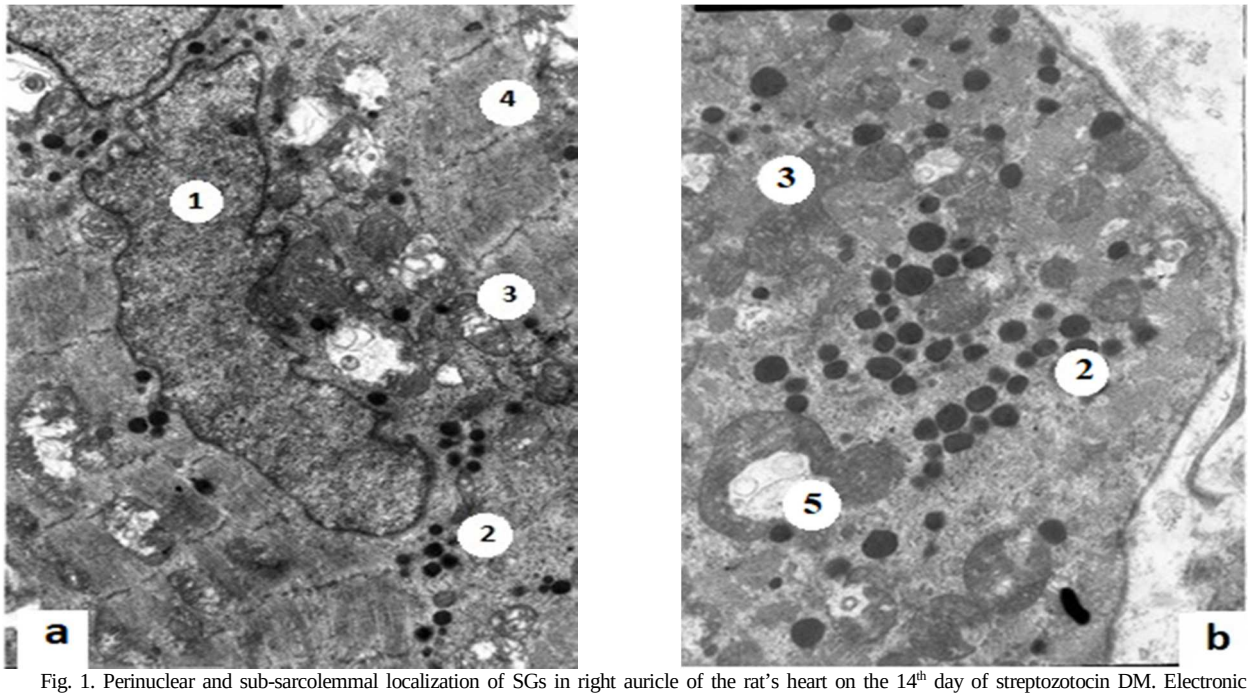

Fig. 1. Perinuclear and sub-sarcolemmal localization of SGs in right auricle of the rat's heart on the 14th day of streptozotocin DM. Electronic microphotographs. Sat.: a) 6400, b) 8000. Markings: 1 – nucleus, 2 – SG, 3 – mitochondria, 4 – myofibrils, 5-vacuoles.

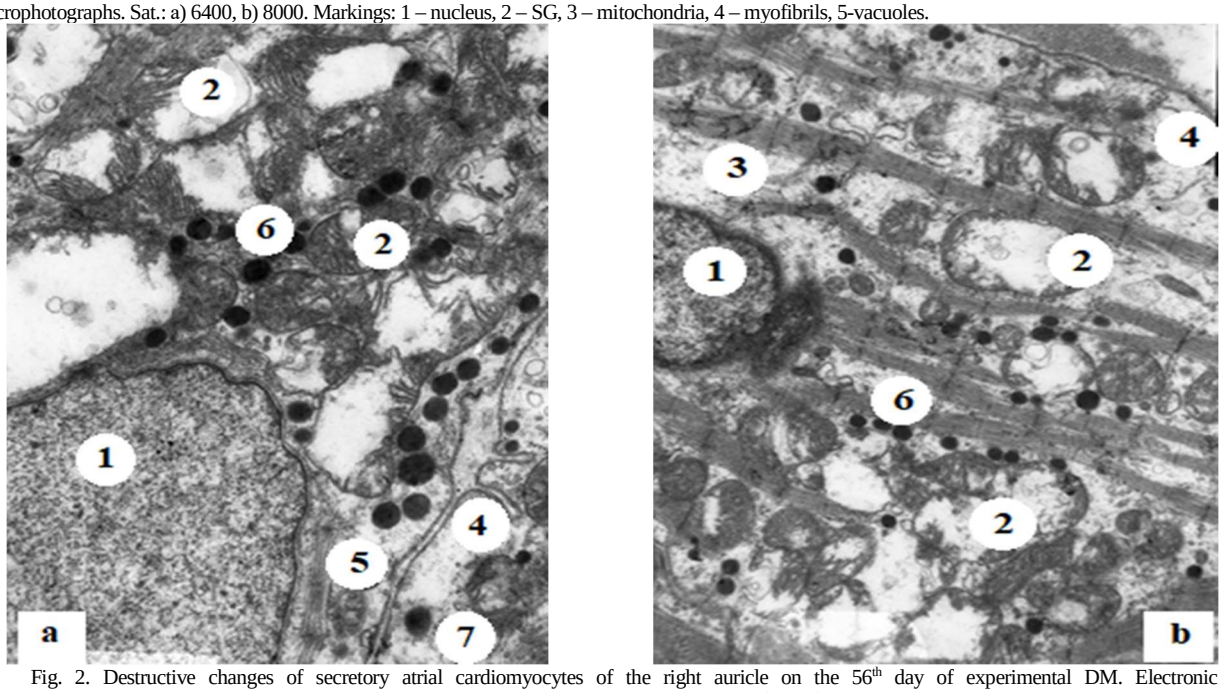

Fig. 2. Destructive changes of secretory atrial cardiomyocytes of the right auricle on the 56th day of experimental DM. Electronic microphotographs. Sat.: a) 9600, b) 6400.Markings: 1 – nucleus, 2 – mitochondria, 3 –lysis of myofilaments, 4 – subsarcolemmal edema, 5 – Golgi complex, 6 – mature SG, 7 – diffusing SG.

Table 1

Bulk Density of Different Types of SG during Experimental Diabetes Mellitus

Part of myocardium	Types of granules	14th day		56th day	
		CG	EG	CG	EG
Right auricle	1-st	1,88±0,09	3,98±0,19**	1,84±0,09	2,56±0,13*,#
	2-nd	3,52±0,18	4,21±0,18	3,48±0,18	3,96±0,20
	3-rd	3,13±0,19	4,38±0,17*	3,09±0,19	4,32±0,19*
	All SG	8,53±0,31	12,57±0,55**	8,41±0,42	10,84±0,57*,#
Left auricle	1-st	0,89±0,08	0,96±0,09	0,87±0,08	0,92±0,07
	2-nd	1,31±0,09	1,27±0,06	1,27±0,09	1,26±0,08
	3-rd	1,97±0,12	2,78±0,14*	1,79±0,08	2,36±0,12*
	All SG	4,17±0,18	5,01±0,25*	3,93±0,18	4,54±0,21*,#

Notes: * p <0.05; ** p <0.01 - probability of indicators in comparison with the previous term of the experiment; # p <0.05; ## p <0.01 - the probability of the indicators compared with the control group.

Such qualitative and quantitative changes in SG, obviously, led to a decrease in the level of ANP in the blood, which was confirmed by data from other researchers [9]. Scientists believe that these changes indicate a failure of compensatory processes [21]. In our opinion, reduction of secretory activity of secretory atrial cardiomyocytes is associated with destructive processes in the cells themselves. Such changes in cardiomyocytes are associated with the development of pronounced diabetic microangiopathy in this period [14].

Conclusions

1. Thus, on the 14th day of the development of experimental DM there was an increase in the functional activity of secretory atrial cardiomyocytes, which morphologically manifested itself: hypertrophy and hyperplasia of the Golgi complex, an increase in bulk density of SGs with the predominance of diffusional forms over young and mature, indicating the enhanced processes of withdrawal of ANP from cells.
2. On the 56th day of experiment, secretory activity of secretory atrial cardiomyocytes decreased due to their exhaustion and the development of destructive processes in them. The bulk density of young and mature SGs decreased, compared to the previous term of the experiment, while that of the diffusing SGs remained high. Such changes in cardiomyocytes develop on the background of diabetic microangiopathy.
3. Future studies of changes in the endocrine system of the heart in experimental diabetes mellitus and its correction with Exenatide are promising. This will allow Exenatide to be recommended or not as an antidiabetic medication for the correction of diabetic cardiomyopathies.

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Реферати

СТРУКТУРНІ ЗМІНИ ЕНДОКРИННОЇ СИСТЕМИ СЕРЦЯ ПРИ СТРЕПТОЗОТОЦИНОВОМУ ЦУКРОВОМУ ДІАБЕТІ

Жураківська О.Я., Микулець Т.И., Голдак У.М., Клипич Я.И., Миськів В.А, Гречин А.Б., Клипич О.О.

Метою роботи було встановлення особливостей структурної перебудови секреторних передсердних кардіоміоцитів у ранні та віддалені терміни перебігу стрептозотоцинового цукрового діабету (ЦД). ЦД моделювали одноразовим внутрішньоочеревинним введенням стрептозотоцину (6 мг на 100г маси тіла). Матеріал для дослідження забирали на 14 та 56 доби експерименту. Використали електронно-мікроскопічний метод дослідження. Встановлено, що стрептозотоциновий ЦД в секреторних передсердних кардіоміоцитах призводить до перебудови внутрішньоклітинних органел, які відповідають за синтез і секрецію передсердного натрійуретичного пептиду (ПНУП). Слід зазначити, що відбувається перерозподіл різних типів секреторних гранул (СГ) у відповідь на гіперглікемію, при цьому на 14 добу експерименту значно збільшується об'ємна щільність дифундуючих СГ, що вказує на посилення процесів виведення ПНУП із клітини, а на 56 добу об'ємна щільність молодих і зрілих СГ достовірно зменшується, що свідчить про зрив компенсаторних механізмів.

Ключові слова: стрептозотоциновий цукровий діабет, щурі, секреторний передсердний кардіоміоцит.

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СТРУКТУРНЫЕ ИЗМЕНЕНИЯ ЭНДОКРИННОЙ СИСТЕМЫ МИОКАРДА ПРИ СТРЕПТОЗОТОЦИНОВОМ САХАРНОМ ДИАБЕТЕ

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Целью работы было определение особенностей структурной перестройки сердечных предсердных кардиомиоцитов в ранние и отдаленные сроки течения экспериментального стрептозотоцинового сахарного диабета (СД). СД моделировали одновременным внутрибрюшинным введением стрептозотоцина (6 мг на 100г массы тела). Материал для исследования изымали на 14 и 56 день эксперимента. Использовали електронно-микрос копический метод исследования. Определено, что стрептозотоциновый СД в секреторных предсердных кардиомиоцитах ведет к перестройке внутриклеточных органэлл, что отвечают за синтез и секрецию предсердного натрийуретического пептида (ПНУП). Следует отметить, что происходит перераспределение разных типов секреторных гранул (СГ) в ответ на гипергликемию, при этом, на 14 сутки експеримента значительно увеличивается обьемная плотность дифундирующих СГ, что указывает на усиление процессов выведения ПНУП из клеток, а на 56 сутки обьемная плотность молодых и зрелых СГ достоверно уменьшается, что свидетельствует о срыве компенсаторных механизмов.

Ключевые слова: стрептозотоциновый сахарный диабет, крысы, секреторный предсердный кардиомиоцит.

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ULTRASTRUCTURE OF ALVEOLAR MACROPHAGES IN CASE OF EXPERIMENTAL ACUTE RENAL FAILURE

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We have done experiments on Vistar line white male rats using electronic microscope method and studied in dynamics (12, 24, 72 hours) the ultrastructural changes of alveolar macrophages in case of experimental acute renal failure. It has been established that already in 12 hours after beginning of the experiment one can observe increase of quantity and functional activity of macrophage cells. With expansion of experimental timeline (24-72 hours), we have observed both dystrophic-destructive and adaptive changes in the alveolar macrophages.

Key words: lung, alveolar macrophages, experimental acute renal failure

The paper is a fragment of RSW "Pathogenetic Development Mechanisms of Changes in the Respiratory, Endocrine, Nervous Systems in Case of Simulated Pathological Conditions and Correction of Thereof" (number of state registration 0117U001758).

It has been established in the multiple clinical and experimental studies that alveolar macrophages (AM) play an important role in support of resistance of human body in case of exposure to exo- and endogenic factors [1, 2, 4, 6]. Analysis of many works has demonstrated that morphofunctional condition of AMs is closely connected with structural and metabolic changes in lungs in case of different pathological conditions [3, 5, 7, 10].

The purpose of this research was to study in dynamics the ultrastructural changes of alveolar macrophages of the respiratory part of lungs in case of experimental acute renal failure (EARF).

Materials and methods. The experiment was done on 45 Vistar line white male rats weighting 180-220 grams, which were subdivided in two groups: control and experimental. Acute renal failure in rats of the experimental group was induced by intramuscular administration of 50% glycerol aqueous solution in quantity of 10 ml per kg of body mass [14]. Equivalent amount of water for injections has been injected to the control group. Lung tissue sampling for electronic microscope examination was done using ketamine anaesthesia in 12, 24, 72 hours after beginning of the experiment. Pieces of lung tissue were fixed in 2,5% solution of gluteraldehyde with further postfixation in 1% solution of osmium tetroxide. After dehydration, the material