**DOI 10.26724/2079-8334-2020-2-72-208-212**
**УДК 616.125.3: 57.012.4:616.37-008.64:57.084.1**

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# FEATURES OF THE RIGHT ATRIUM STRUCTURE IN EXPERIMENTAL DIABETES MELLITUS AND USE OF ANTIOXIDANTS

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Right atrium cardiomyocytes were studied in conditions of streptozotocin-induced type 1 diabetes mellitus and with the use of N-acetylcysteine as an antioxidant. The study showed that potential damage of fine mechanisms of synthesis and secretion of atrial natriuretic peptide may be the cause of cardiovascular pathology in diabetes. Use of antioxidant drugs has revealed that N-acetylcysteine partially reduces cardiomyocyte swelling, increases the number of atrial granules containing atrial natriuretic peptide and stimulates autophagy. Based on ultrastructural studies of atrial cardiomyocytes in type 1 diabetes it was found that damage to protein synthesis and accumulation and transformation of abnormal proteins happen via their elimination in two ways: through the ubiquitin-proteasomal and autophago-lysosomal systems. Autophagosomes occur both with a double membrane, i.e. the newly formed one, and with a single membrane after contact with lysosomes. These processes take place to maintain homeostasis in cardiomyocytes and in the myocardium as a whole. Impairement of these systems may lead to development of diabetic cardiomyopathy.

**Key words**: type I diabetes mellitus in rats, right atrium cardiomyocytes, atrial granules, autophagy.

*The work is a fragment of the research project "Study of cellular and molecular mechanisms of pharmacological influence on the reprogramming of macrophages functional phenotype in wound regeneration in the presence of hyperglycemia", state registration No. 0119U1011219.*

Cardiovascular complications are often the cause of death of patients with diabetes mellitus (DM). Acute hyperglycemia is associated with heart failure and cardiogenic shock [5]. In the study of this issue preference is given to the left ventricle [2]. Currently, given the endocrine function of the heart, which is mainly performed by the right atrium, and possible complications of diabetes the state of atrial cardiomyocytes attracts considerable interest. In recent years, the role of secretory granules, the morphological equivalent of atrial natriuretic peptide (ANP), in the development of chronic heart failure has been actively studied. The role of ANP in diabetes mellitus, and in pathological complications accompanied by hyperglycemia-induced oxidative stress is unknown. The latter leads to the formation of abnormal cellular proteins that are eliminated through the ubiquitin-proteosomal pathway or by autophagy. In violation of these mechanisms, the risk of deterioration of cardiac function increases. Disruption of these mechanisms increases the risk of cardiac function deterioration. It is known that aberrant proteins and destroyed organelles are engulfed by autophagosomes and destroyed in lysosomes [4]. At the molecular level DM was found to initiate apoptosis of cardiomyocytes by reducing cardiac autophagy, which in fact causes the development of diabetic cardiomyopathy [8]. Impairement of these mechanisms increases the risk of cardiac function deterioration. This indicates the key role of oxidative stress in the regulation of autophagy and the possibility of using drugs with antioxidant action to modulate cardioprotective autophagy with the aim of preventing and correcting diabetic cardiomyopathy. [3,4].

The antioxidant drug N-acetylcysteine (NAC) attracts significant attention. In an ischemia/reperfusion experimental model NAC has significantly reduced induced oxidative stress and cardiomyocyte apoptosis thus preventing postischemic autophagy in DM. [9]. In our earlier studies we have found a positive effect of N-acetylcysteine on heart rate, ventricular contractile activity, and signs of hypertrophy in rats in the early stage of diabetic cardiomyopathy in the left ventricle. [2]. Currently the question of whether NAC will demonstrate a cardioprotective effect on ANP and activate or inhibit autophagy and apoptosis in rats in a model of diabetic cardiomyopathy remains unresolved?

**The purpose** of the study was to investigate the features of the right atrium cardiomyocytes ultrastructure in conditions of streptozotocin-induced type 1 diabetes mellitus and the use of N-acetylcysteine as an antioxidant.

**Materials and methods**. Type 1 diabetes mellitus (DM) was simulated by administration of 50 mg / kg of streptozotocin (STZ) to rats. *General Ethical Principles for Animal Experiments* approved by the 1st National Congress on Bioethics (Kyiv, 2001) have been observed throughout the study. Experimental animals were divided into 3 groups: 1 - control group (intact rats receiving 0.9% saline); 2 - DM1 (a group of animals with DM1 simulated by streptozotocin administration); 3 - NAC (group of diabetic rats receiving N-acetylcysteine at a dose of 1.5 g/kg per os). Used as the material for electron microscopic examinations were pieces of the right atrium myocardium, which were fixed with 2.5% solution of glutaraldehyde on phosphate buffer with postfixation in 1% solution of osmium tetroxide and

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treated according to conventional methods. Sections were made on an ultramicrotome LKB III (Sweden). The preparations were studied and photographed on an electron microscope PEM-125K.

**Results of the study and their discussion**. The study revealed that under experimental type 1 diabetes mellitus cardiomyocytes in the right atrium myocardium demonstrate both cytoplasmic swelling and hyperosmia, local myofibril hypercontraction, and increased number of agranular tubules of the endoplasmic reticulum in comparison with control. An accumulation of collagen fibers was found in the interstitium. Ultrastructural analysis of atrial cardiomyocytes showed their heteromorphic structure in simulated diabetes mellitus. In some of them cytoplasmic swelling, myocytolysis, disorganization of myofibrils, compaction of intercalated discs and mitochondria and destruction of their cristae were found (fig. 1A). Other cardiomyocytes preserved structural organization of myofibrils and mitochondria, but cardiomyocytes in the pre-apoptotic state also occurred (fig.1B). Chromatin in the nuclei of such cells was located only near the nuclear envelope, the integrity of which was lost with cytoplasmic contents (lysosomes, destructively altered mitochondria, autophagosomes) found in the center of the nucleus. In the cytoplasm of atrial cardiomyocytes in the context of diabetes, autophagosomes were observed at different stages of formation: with a double membrane (primary), or a single (secondary) one and electron-dense contents (mostly protein), and in contact with lysosomes.

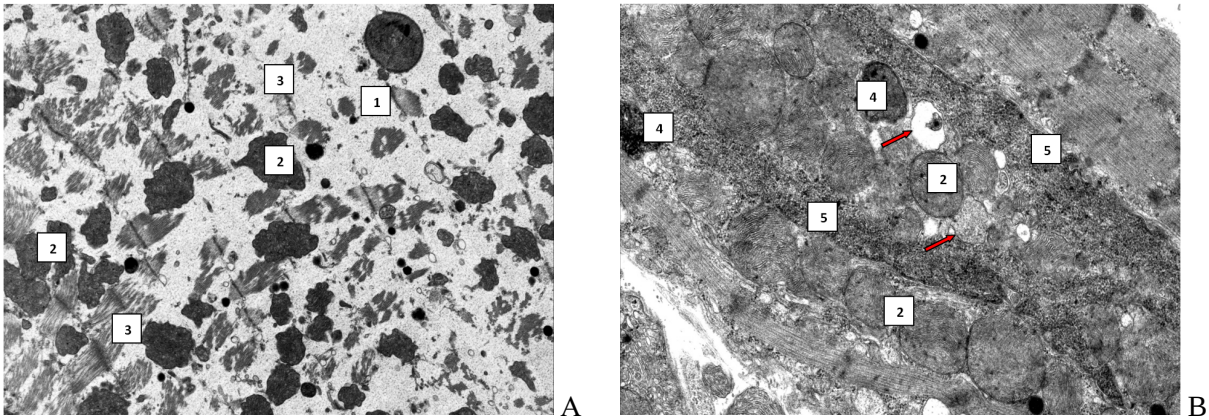



Fig. 1. A - a fragment of an atrial cardiomocyte in the context of diabetes mellitus: 1 - cytoplasmic swelling, 2 - mitochondria, 3 - myofibrils. Magnification: 10000; B - fragment of an atrial cardiomyocyte: 4 - lysosomes, ↑ - autophagosomes, 5 - nuclear apoptosis. Magnification: 20000

The sarcolemma of such cells is partially lysed, as evidenced by the detection of individual mitochondria in the interstitium.

Autophagosomes were observed in cardiomyocytes in quite a large number. The latter were detected both with a double membrane, i.e. the newly formed (primary) and with a single one (secondary), after contact with lysosomes. The number of autophagosomes increases compared to the control, some of them contain cellular detritus, or individual organelles, such as mitochondria (fig.2 A, B).

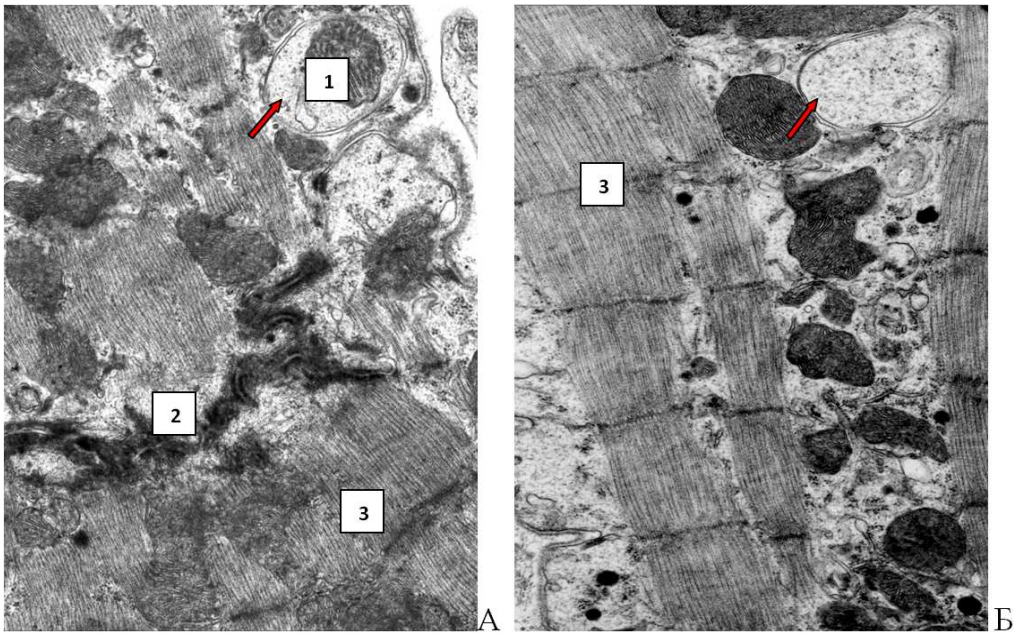



Fig. 2. Fragments of cardiomyocytes of the right atrium in conditions of diabetes mellitus. ↑ - primary autophagosomes, 1 - mitochondria in the autophagosome, 2 - intercalated disc, 3 - myofibrils. Magnification: 18000

The nuclei of some cells had deep invaginations, which revealed cytosigresomes containing cellular detritus, a large number of vacuoles and autophagosomes. (fig.3). Accumulation of abnormal proteins in the cytoplasm of atrial cardiomyocytes in diabetes mellitus is due in part to impaired atrial natriuretic peptide secretion. Concurrently large cytosigresomes including both autophagosomes and elements of the ubiquitin-proteasomal system were observed, which eliminated the damaged (aberrant) intracellular proteins. (fig. 3).

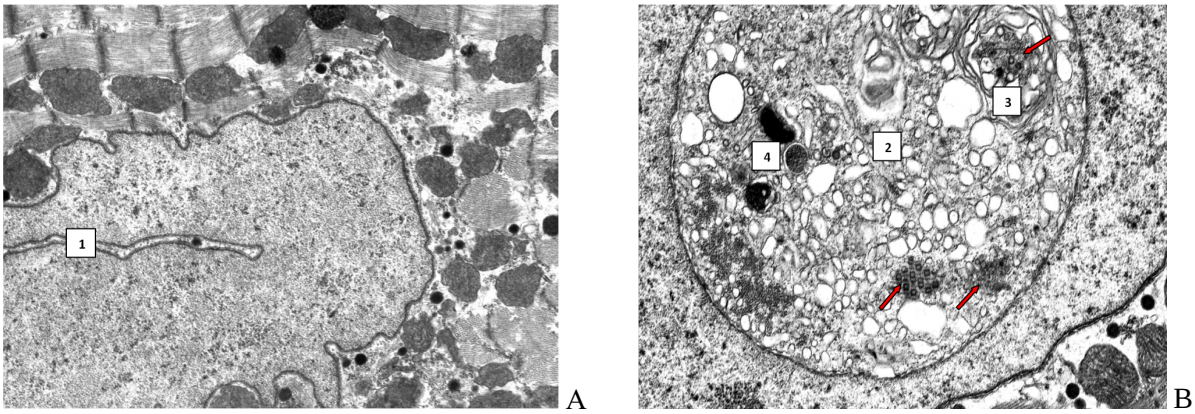



Fig. 3. Fragments of the right atrium cardiomyocytes in conditions of diabetes mellitus. 1 - invagination of the nuclear membrane, 2 - cytosigrisome, 3 - autophagosomes, 4 - lysosomes, ↑ - proteasomes. Magnification: A - 10000, B - 20000.

The electron micrograph shows the cytosigresome in the invagination of the nuclear envelope, which contains elements of cellular structures: vacuoles of different sizes, lysosomes, autophagosomes and complexomixes, which seem to be proteasomes equivalents. They include microtubules formed for ubiquitination, after which they break down, while amino acids seem to be used again.

Simulation of diabetes mellitus in the right atrium myocardium revealed a few atrial granules containing ANP, as compared with the control (see fig. 1, 2, 3). In the perinuclear zone, where these granules are formed in the Golgi complex, some swelling and granules of type I too small in size are found. They are newly formed and present in small quantity. Single granules are located between myofibrils. The density of the atrial granules is much higher than in the control, which seems to be due to the condition of the granules themselves. They are mainly type 1 (high density) and are not released into the bloodstream, which may be the result of increased pressure in it. Fewer type 3 granules (diffusing) are found in diabetes mellitus. It is obvious that changes in the synthesis and secretion of such a strong vasodilator as ANP can be a cause of diabetes complications.

The use of antioxidants has shown that N-acetylcysteine partially reduces the swelling of cardiomyocytes. In addition to the typical nuclei of cardiomyocytes, there occur nuclei with invaginations of the nuclear membrane. The number of autophagosomes is increased as compared with animals with simulated diabetes. Most autophagosomes have a double membrane, they are located both in the perinuclear zone and subsarcolemmally and are formed by different membrane structures: endoplasmic reticulum tubules that have lost ribosomes, Golgi cisterns, plasma membrane, and parts of intercalated discs (fig. 4).

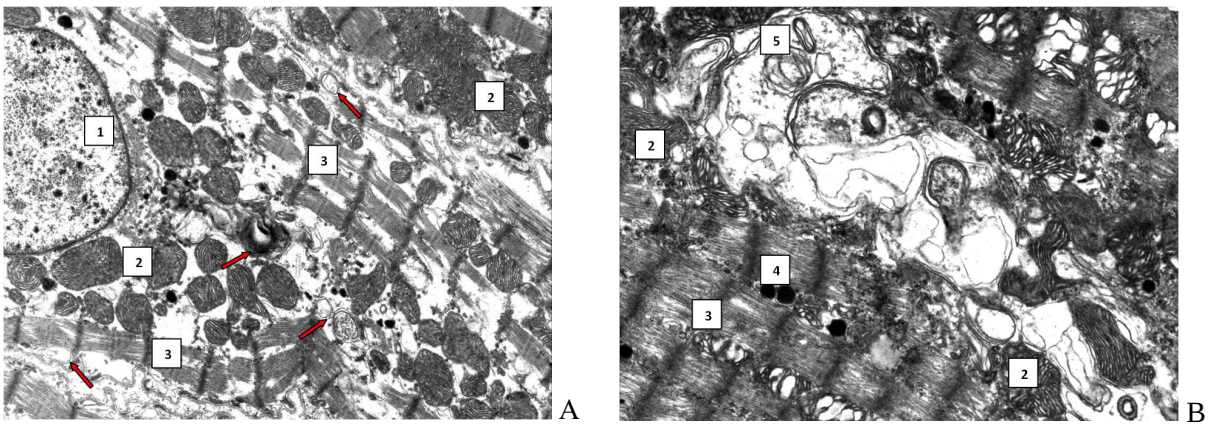



Fig. 4. Fragments of the right atrium cardiomyocytes in conditions of diabetes mellitus during the use of N-acetylcysteine. A: 1 - nucleus, ↑ - autophagosomes, 2 - mitochondria, 3 - myofibrils. B: 4 - atrial granules, 5 - clusters of mitosomes. Magnification: A - 12000, B - 14000.

A special type of autophagosomes - mitosomes are formed by mitochondrial membranes. In the zone of mitosome formation the latter have a hyperosmic matrix, if it is preserved, or a vacuolated

intercristal space. Atrial granules containing ANP are localized perinuclearly in the Golgi complex area, they are different in size and mainly belong to type 1.

Thus, the conducted study showed that possible damage to the fine mechanisms of synthesis and secretion of atrial natriuretic peptide may be the cause of cardiovascular pathology in diabetes mellitus. The use of antioxidant drugs revealed that N-acetylcysteine partially reduces the swelling of cardiomyocytes, increases the number of atrial granules containing ANP and stimulates autophagy. Based on the conducted ultrastructural studies of atrial cardiomyocytes in type 1 diabetes mellitus and literature data, it can be assumed that damage of protein synthesis and accumulation and cleavage of abnormal proteins occur by their elimination in two ways: through ubiquitin-proteasomal and autophagous-lymphatic systems. [1, 2, 3]. Autophagosomes occur both with a double membrane, i.e. the newly formed ones, and with a single membrane after contact with lysosomes. These processes take place to maintain homeostasis in cardiomyocytes and in the myocardium as a whole. Impairement of these systems may lead to the development of a diabetic cardiomyopathy [7].

The tubular complexomixes found in cytosegresomes, which are analogs of protesomes may serve confirmation of these processes. The authors describe them as a radial symmetric protein complex consisting of 26 subunits that form four 7-membered rings packed on top of one another [1]. Hyperglycemia-induced oxidative stress accompanying diabetes leads to the formation of damaged cellular proteins that must be eliminated through the ubiquitin-proteasomal pathway or autophagy. This process - utilization of proteasomes through autophagy is called "proteaphagy". Impairement of these mechanisms increases the risk of cardiac function deterioration [8]. Large areas of mitophagosomes found by us, which are signs of mitochondrial autophagy, indicate the activation of mitophagia as a response to oxidative stress caused by diabetes. According to some authors, mitophagia is responsible for the elimination of damaged mitochondria [6]. This indicates the key role of oxidative stress in the regulation of autophagy and the possibility of using drugs with antioxidant action for prevention and correction of diabetic cardiomyopathy.

## Conclusion

The conducted ultrastructural study of atrial cardiomyocytes in type 1 diabetes showed that impairement of protein synthesis as well as accumulation and transformation of abnormal proteins happen via their elimination in two ways: namely, through the ubiquitin-proteasomal and autophago-lysosomal systems. Autophagosomes occur with both a double membrane, i.e. the newly formed ones, and with a single membrane after their contact with lysosomes. These processes take place to maintain homeostasis in cardiomyocytes and in the myocardium as a whole. The antioxidant drug -N-acetylcysteine partially reduces the swelling of cardiomyocytes, increases the number of atrial granules containing ANP and stimulates autophagy and mitophagia.

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## Реферати

**ОСОБЛИВОСТІ БУДОВИ ПРАВОГО ПЕРЕДСЕРДЯ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ЦУКРОВОМУ ДІАБЕТІ ТА ЗА УМОВ ЗАСТОСУВАННЯ АНТИОКСИДАНТІВ**

**Стеченко Л.О., Чайковський Ю.Б., Кривошеєва О.І., Чухрай С.М., Пастухова В.А., Ірха С.В.**

Досліджувались кардіоміоцити правого передсердя за умов стрептозотоцин-індукованого цукрового діабету 1

**ОСОБЕННОСТИ СТРОЕНИЯ ПРАВОГО ПРЕДСЕРДИЯ ПРИ ЕКСПЕРИМЕНТАЛЬНОМ САХАРНОМ ДИАБЕТЕ И В УСЛОВИЯХ ПРИМЕНЕНИЯ АНТИОКСИДАНТА**

**Стеченко ЛА., Чайковський Ю.Б., Кривошеєва О.И., Чухрай С.Н., Пастухова В.А., Ірха С.В.**

Исследовались кардиомиоциты правого предсердия при стрептозотоцин-индуцированом сахарном диабете 1

типу та при застосуванні N-ацетилцистеїну як антиоксиданта. Проведене дослідження показало, що можливе, ушкодження тонких механізмів синтезу та секреції передсердного натрійуретичного пептиду може бути причиною виникнення серцево-судинної патології за умов цукрового діабету. Застосуванням препаратів антиоксидантної дії було встановлено, що N-ацетилцистеїн частково зменшує набряк кардіоміоцитів, підвищує кількість передсердних гранул, що містять передсердний натрійуретичний пептид та стимулює автофагію. Базуючись на проведених ультраструктурних дослідженнях передсердних кардіоміоцитах при цукровому діабеті першого типу, встановлено, що ушкодження білкового синтезу та накопичення і трансформація аномальних білків відбувається елімінацією їх двома шлях: через убіквитин-протеасомальну та автофаго-лізосомальну системи. Автофагосоми трапляються як з подвійною мембраною тобто новоутворені, так і з одинарною після контакту з лізосомами. Ці процеси здійснюються для підтримки гомеостазу у кардіоміоцитах та у міокарді в цілому. При порушенні цих систем можливий розвиток діабетичної кардіоміопатії.

**Ключові слова:** кардіоміоцити правого передсердя, цукровий діабет у щурів, автофагія

Стаття надійшла 15.06.2019 р.

типа и в условиях применении N-ацетилцистеїна, как антиоксиданта. Проведенное исследование показало, что возможное, повреждение тонких механизмов синтеза и секреции предсердного натрійуретического пептида может быть причиной развития сердечно-сосудистой патологии в условиях сахарного диабета. Применение препарата антиоксидантного действия показало, что N- ацетилцистеїн частично уменьшает отек кардиомиоцитов, повышает количество предсердных гранул, содержащих предсердний натрийуретический пептид и стимулирует автофагию. Основываясь на проведенных ультраструктурных исследованиях предсердних кардиомиоцитов при сахарном диабете первого типа, установлено, что повреждение белкового синтеза, накопление и трансформация аномальных белков осуществляется элиминацией их двумя путями: через убиквитин-протеасомальний и автофаго-лизосомальную системы. Автофагосоми обнаруживаются как с двойной мембраной, то есть новообразованные, так и с одинарной после контакта с лизосомами. Эти процессы осуществляются для поддержки гомеостаза у кардиомиоцитах и в миокарде в целом. При нарушение этих систем возможное развитие диабетической кардиомиопатии.

**Ключевые слова:** сахарный диабет 1 типа у крыс, кардиомиоциты правого предсердия, предсердные гранулы, автофагия.

Рецензент Єрошенко Г.А.