Ultrastructural changes in the myocardium of animals under conditions of simulated hyperhomocysteinemia, hyper- and hypothyroidism and their combination

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Thyroid hormones have a significant impact on heart function through both genomic and non-genomic effects. Deficiency or excess of thyroid hormones leads to profound changes in the regulation of cardiac function and cardiovascular hemodynamics. The heart is the main target organ for the action of thyroid hormones and in patients with hypo- or hyperthyroidism there are marked changes in the work of the heart. The aim of the work was to establish ultrastructural changes in myocardial components in experimental hyperhomocysteinemia (HHCy) against the background of hyper- and hypothyroidism. Thiolactone HHCy was modelized by administering to animals an exogenous HCy in the form of thiolactone at a dose of 100 mg/kg body weight once a day for 28 days. Hyperthyroidism was modelized by daily administration of L-thyroxine at a dose of 200 μg/kg for the 21 days, hypothyroidism - daily administration of thiamazole at a dose of 10 mg/kg for the 21 days. Individual groups of animals were administered L-thyroxine and thiamazole in parallel with HCy. High levels of HCy adversely affected the walls of myocardial blood vessels. The lumens of hemocapillaries were plethoric, filled with erythrocytes. Changes in endotheliocytes were revealed, and cardiomyocytes contained deformed nuclei. In laboratory animals with hyperthyroidism, an increase in ultrastructural changes in the walls of blood vessels (edema of the walls of hemocapillaries, damaged cristae in mitochondria) were established. In animals that were modeled for hyperthyroidism and HHCy, more significant changes in endotheliocytes were revealed, most of the mitochondria were destroyed. More pronounced alterative changes were revealed in cardiomyocytes. An electron microscopic examination of the myocardium of animals with hyperthyroidism showed significant degenerative changes in the ultrastructure of the walls of blood vessels, and hypertrophied mitochondria were also found. The combined influence of hyperthyroidism and HHCy caused the most profound disturbances in the ultrastructure of cardiomyocytes and hemocapillaries in comparison with other groups of animals. The integrity of intercellular contacts was impaired, most of the mitochondria of myocytes had destroyed cristae and the outer membrane.

Keywords: hyperthyroidism, hypothyroidism, hyperhomocysteinemia, myocardium.

Introduction
Thyroid hormones are known to affect the cardiovascular system [18]. Clinically, both excess and deficiency of thyroid hormones may cause or exacerbate cardiovascular disorders, including atrial and ventricular arrhythmias, atherosclerotic vascular disease, dyslipidemia, and heart failure, thereby increasing the risk of premature death and disease. Thyroid hormones are an important regulator of cardiac function and cardiovascular hemodynamics. Triiodothyronine (T₃) binds to nuclear receptor proteins and mediates the expression of several important cardiac genes, causing transcription of positively regulated genes, including β-myosin heavy chain and sarcoplasmic reticulum calcium ATPase. Negatively regulated β-myosin and phospholamban genes are reduced in the presence of normal levels of thyroid hormone in the serum [16]. T₃-mediated effects on the cardiovascular system include relaxation of vascular smooth muscle, leading to decreased arterial resistance.
and diastolic blood pressure. With hyperthyroidism, heart rate and cardiac output increase, and systemic vascular resistance decreases, whereas with hypothyroidism, the opposite is true.

Changes in plasma or tissue levels of thyroid hormones are associated with significant changes in cardiovascular function. A significant proportion of patients with heart failure have some form of thyroid dysfunction, including hypothyroidism, hyperthyroidism and low T₃ syndrome [8]. Several clinical and experimental studies have shown the beneficial effects of thyroid hormones in cardiac pathology [2, 6, 19]. Epidemiological data confirm a higher risk of heart failure and a worse prognosis in patients with low thyroid hormone heart failure.

The aim of the study was to establish ultrastructural changes in myocardial components under the conditions of simulated HHCy, hyper- and hypothyroidism and their combined effects.

Materials and methods

The experiments were performed on 50 outbred white male rats weighing 180-200 g. Rats were kept at standard light day on a normal diet. All studies were conducted in compliance with the requirements of humane treatment of experimental animals, regulated by the Law of Ukraine "On Protection of Animals from Cruelty" (№ 3447-IV of 21.02.2006) and the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, March 18, 1986). The Committee on Bioethics of National Pirogov Memorial Medical University, Vinnytsya found that the study does not contradict the basic bioethical standards (Minutes № 7 of 7.04.2022).

All animals were divided into 5 groups: 1 - intact rats. This group of animals was injected intragastrically with 1 % starch solution; 2 - animals with thiolactone HHCy, which was caused by intragastric administration of HCy in the form of thiolactone at a dose of 100 mg/kg body weight in 1 % starch solution once a day for 28 days. The dose, route and duration of thiolactone administration of HCy are borrowed from the literature and did not cause animal death [14]; 3 - animals with hyperthyroidism, which were administered intragastrically daily for 21 days L-thyroxine at a dose of 200 μg/kg in 1 % starch solution [11]; 4 - animals with thiolactone HHCy, which were daily administered intragastrically daily for 21 days L-thyroxine at a dose of 200 μg/kg in 1 % starch solution; 5 - animals with hypothyroidism, which were daily administered intragastrically on the 21st day mercazolyl in 1 % starch solution at a dose of 10 mg/kg body weight [11]; 6 - animals with thiolactone HHCy, which were administered intragastrically mercazolyl at a dose of 10 mg/kg per 1 % starch solution daily for 21 days. Animals were removed from the experiment 24 hours after the last administration of the selected substances.

Collection of material for electron microscopic examination of the myocardium was performed according to generally accepted rules [4]. The material was fixed in 2.5 % glutaraldehyde solution with active reaction medium pH 7.2-7.4, prepared on phosphate buffer. Postfixation was performed with 1 % osmium tetroxide solution, followed by dehydration in alcohols and propylene oxide and poured into a mixture of epoxy resins. Ultrathin sections made on an ultramicrotome LKB-3 (Sweden) were stained with 1 % aqueous uranyl acetate solution, contrasted with lead citrate according to the Reynolds method and studied under an electron microscope PEM-125K.

Results

Ultrastructural studies of the heart wall of intact animals have shown that cardiomyocytes have an elongated shape and are interconnected by desmosomal and slit contacts. Externally, cardiomyocytes are surrounded by sarcolemma. In the central part of the cells is an oval nucleus with one or two nucleoli. Euchromatin predominates in the karyoplasm. The karyolemna contains a uniform perinuclear space and numerous nuclear pores. In the cytoplasm of cells there are organelles of general and special purpose (myofibrils). There is a large number of mitochondria, which are located between myofibrils in the form of chains and groups near the nucleus. These organelles contain densely packed cristae and electron-dense matrix. In the perinuclear part of the cytoplasm there are tanks of the Golgi complex, free ribosomes and polyribosomes. The smooth endoplasmic reticulum forms a system of anastomotic tubules and contacts the T-tubes. A significant amount of cytoplasm is occupied by myofibrils. In the longitudinal section of the cell, they have a well-defined sequential alternation of actin and myosin microfilaments in the sarcomeres (Fig. 1). Between the myocardial muscle fibers is a stromal loose connective tissue that contains blood vessels and nerves.

The myocardium contains a significant number of...
hemocapillaries. The capillary wall is formed by endothelial cells that include the oval nucleus. Karyoplasm contains mainly euchromatin, heterochromatin forms small lumps, nucleoli are rare. The cytoplasm of endothelial cells has a moderate electron density, organelles are moderately developed and localized near the nucleus. Micropinocytic vesicles are found in peripheral, cytoplasmic areas. Endothelial cells lie on a clearly contoured solid basement membrane, in its cleavage or outside localized pericytes and adventitial cells, which are rare. The lumen of hemocapillaries is moderate (Fig. 2).

Submicroscopic studies of the heart of laboratory animals of the second experimental group, which simulated HHCy, showed the presence of alternative changes in the walls of blood vessels. The lumens of the hemocapillaries were mostly full-blooded, mostly filled with erythrocytes.

Changes in endothelial cells were revealed, which were manifested by edema of the peripheral part of the cell, a decrease in the number of pinocytic vesicles and caveolae, an increase in heterochromatin in the nucleus and local expansion of the perinuclear space. Contacts between endothelial cells were partially disrupted. The basement membrane is compacted or locally swollen (Fig. 3).

Impaired microcirculation of the organ provoked changes in cardiomyocytes. In the deformed nuclei of cardiomyocytes the growth of intussusception of a karyolemma is revealed, in their karyoplasm heterochromatin prevails, nucleoli are not defined. Hypertrophied mitochondria with electron-dense matrix and reduced cristae were observed in electron-dense sarcoplasm. In some cells, myofibrils were shortened, compacted, homogenized, and sarcomere structure was

![Fig. 2. Ultrastructural state of the hemocapillary of an intact group animal. Cardiomyocyte fragment (1), capillary lumen (2) with erythrocyte, nucleus (3) and endothelial cell cytoplasm (4), basement membrane (5), perivascular space (6). x1 4000.](image)

![Fig. 3. Ultrastructural changes in the hemocapillary of the left ventricular myocardium of the animal under conditions of simulated HHCy. Capillary lumen with erythrocytes (1), deformed nucleus (2), swollen cytoplasmic area of endothelial cell (3), basement membrane (4), fragment of cardiomyocyte (5). x1 4000.](image)

![Fig. 4. Ultrastructural changes of the cardiomyocyte of the left ventricle of the animal under the conditions of simulated hyperhomocysteinemia. Deformed nucleus (1), destructively altered mitochondria with fragmented cristae (2), myofibril overgrowth zone (3), myofibril lysis sites (4). x15 000.](image)

![Fig. 5. Ultrastructural changes of the left ventricular myocardium in simulated hyperthyroidism. Capillary lumen with erythrocytes (1), endothelial cell cytoplasm (2), basement membrane (3), cardiomyocyte fragment (4). x14 000.](image)
Submicroscopic studies of the heart of laboratory animals of the third experimental group, which simulated hyperthyroidism, revealed edema of the walls of hemocapillaries, while the basement membrane was thickened, the cytoplasm of endothelial cells was enlightened. The lumenal surface of endothelial cells formed single cytoplasmic outgrowths and microvilli, and a decrease in micropinocytic vesicles in the cytoplasm was determined. In some mitochondria the cristae were damaged, the tubules of the endoplasmic reticulum were dilated (Fig. 5).

Changes in the ultrastructural organization of cardiomyocytes were manifested by increased intussusception of the karyolemma and expansion of the perinuclear space. An increase in changes in the structural components of the sarcoplasm of some myocytes was revealed. Myofibrils were thinned with partial lysis, in some areas sarcomeres were located in a disorderly manner, areas of myofibril shortening were determined. Enlightenment of the matrix and damage to the cristae were found in the mitochondria. The tubules of the sarcoplasmic reticulum are dilated (Fig. 6).

Studies of ultrastructural reorganization of the heart of laboratory animals of group IV, which simulated hypothyroidism and HHCy, revealed hemocapillaries with narrowed lumens filled with platelets and erythrocytes. Endothelial cells underwent significant changes. Their nuclei were reduced, there were no nucleoli in the karyoplasm, the karyolemma formed deep

submicroscopic changes of the cardiomyocyte of the left ventricle in white rat under the conditions of simulated hyperthyroidism. Nucleus (1), altered mitochondria (2), zones of myofibril rupture (3) and lysis (4), dilated tubules of the endoplasmic reticulum (5). x14 000.

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Submicroscopic changes of the hemocapillary of the left ventricular myocardium of the animal under conditions of simulated hyperthyroidism and HHCy. Capillary lumen with erythrocytes (1), swollen cytoplasmic area of endothelial cell (2), basement membrane (3), fragment of cardiomyocyte (4). x20 000.

Submicroscopic changes of myocardial cardiomyocyte of the left ventricle of white rat heart under conditions of simulated hyperthyroidism and HHCy. Damaged mitochondrial membranes (1), myofibril lysis zones (2), optically bright areas of the cell, devoid of organelles (3). x18 000.

Ultrastructural changes of the left ventricular myocardial hemocapillary of a white rat heart under the conditions of simulated hypothyroidism. Erythrocyte stasis in the capillary lumen (1) endothelial cell cytoplasm (2), thickened basement membrane (3), cardiomyocyte fragment (4). x14 000.
intussusception. The cytoplasm of endothelial cells is enlightened and swollen. The tubules of the endoplasmic reticulum are dilated and partially fragmented. In most mitochondria, cristae and the matrix were significantly enlightened. Few pinocytic vesicles were found in the cytoplasm. The basement membrane of hemocapillaries is thickened and poorly contoured (Fig. 7).

Cardiomyocytes showed more pronounced alternative changes compared to previous observation groups. A decrease in the area of the nucleus, an increase in the number and depth of karyolemma intussusception was found. Karyoplasm was filled with homogeneous chromatin, nucleoli were not detected. Disturbances in myofibrils were manifested by their thinning, fragmentation and lysis. In most mitochondria the cristae were destroyed, in some of these organelles the outer membrane was damaged. Sarcoplasm of cells is swollen, enlightened, electron-bright areas devoid of organelles are determined (Fig. 8).

Electron microscopic examination of the myocardium of laboratory animals of group V, which simulated hypothyroidism, found that hemocapillary endothelial cells contained few microbubbles and caveolae, their cytoplasm was swollen. It revealed dilatation of the endoplasmic reticulum tubules, hypertrophied mitochondria with an enlightened matrix and partially reduced cristae. The karyolemma of the nuclei formed deep intussusception, the perinuclear space was unevenly expanded. Thickening and swelling of the basement membrane of blood vessels were detected (Fig. 9).

Decreased levels of thyroid hormones caused a violation of the ultrastructure of contractile cardiomyocytes. Euchromatin predominated in the nuclei, heterochromatin formed osmophilic lumps, nucleoli were rare. Karyolemma formed deep intussusception. Sarcoplasm revealed damage to the contractile apparatus of the cell, which was manifested by stratification of myofibrils and their partial fragmentation or compaction, homogenization, areas of overshortening were detected. Sarcomeres were of different sizes, actin and myosin microfilaments were located unevenly. Most mitochondria were hypertrophied, their matrix was enlightened, cristae were partially destroyed, not clearly contoured. The expansion of the tubules and vacuoles of the endoplasmic reticulum was determined (Fig. 10).

Submicroscopic studies of laboratory animals heart of the VI experimental group revealed profound violations of the ultrastructure of endothelial cells. The nuclei of endothelial cells were compacted, pyknotically altered in some cells. The cytoplasm was clear and swollen,
micropinocytic vesicles were rare. The lumenal part of the plasmalemma is indistinctly blurred or destroyed. The integrity of intercellular contacts is violated. The tubules of the endoplasmic reticulum were dilated and partially fragmented. In many mitochondria, cristae and outer membranes were destroyed. The mitochondrial matrix is enlightened. The integrity of intercellular contacts is violated. The basement membrane of the blood vessel wall is significantly thickened and poorly contoured, damaged (Fig. 11).

An increase in ultrastructural changes was also found in cardiomyocytes. The cell nuclei were compacted and pyknotically altered, the perinuclear space was weakly expressed. Mitochondria were hypertrophied, they revealed violations of the integrity of the outer and inner membranes. Myofibrils have undergone profound changes. Thinning, fragmentation, and lysis were observed in most cells, and sarcomeres were unevenly distributed. Activation of fibroblasts is accompanied by the growth of collagen fibers in the interstitium (Fig. 12).

Discussion
The results of submicroscopic examination of the myocardium in HHCy are consistent with the available data from the literature. We found a negative effect of HCY on the walls of myocardial blood vessels. The lumens of the hemocapillaries were mostly full-blooded, mostly filled with erythrocytes. Changes in endothelial cells were detected, myocytes contained deformed nuclei. There are several theories that can explain our data. HCY is thought to cause degradation and block the synthesis of "long-lived" peptides such as collagen, elastin and protein glycans. It is able to cause destruction of bridges in proteins, which leads to their toxic effects, which leads to changes in myocyte energy production (oxidative phosphorylation, glycolysis), intracellular synthesis (protein synthesis) and contractile function of myofibrils.

Our electron microscopic examination of the myocardium of animals with hypothyroidism showed significant degenerative changes in the ultrastructure of blood vessel walls. Hemocapillary endothelial cells contained few microbubbles and caveolae, and their cytoplasm was swollen. Hypertrophied mitochondria with an enlightened matrix and partially reduced cristae were detected. Thickening and swelling of the basement membrane of blood vessels, hypothyroidism caused a violation of the ultrastructure of contractile cardiomyocytes. Work of Udovcic M. and others [15] have shown that hypothyroidism is associated with decreased cardiac output due to impaired vascular smooth muscle relaxation and decreased endothelial nitric oxide availability. This causes a cascading effect of increasing the stiffness of the arteries, which leads to increased systemic vascular resistance. At the molecular level, these changes are the result of decreased Ca²⁺-ATPase expression of the sarcoplasmic reticulum and increased expression of phospholamban, which inhibits ATPase. Thyroid hormones also affect the renin-angiotensin-aldosterone system. Under the action of T₃, renin substrates are synthesized in the liver. Thus, in the hypothyroid state, diastolic blood pressure increases, pulse pressure decreases, and renin levels decrease. This leads to diastolic hypertension, which is often sensitive to sodium. Thyroid hormones also regulate pacing genes through transcription, as well as...
the beta-adrenergic system in cardiomyocytes. As a result of these mechanisms, heart rate increases in the presence of thyroid hormones and decreases in hypothyroidism [17].

Under the conditions of the combined influence of hypothyroidism and HHCy, we found the deepest violations of the ultrastructure of endothelial cells. Integrity of intercellular contacts is broken, in many mitochondria cristae and an external membrane were destroyed. The basement membrane of the blood vessel wall is significantly thickened and poorly contoured, damaged. An increase in ultrastructural changes was also found in cardiomyocytes (hypertrophied mitochondria). Thinning, fragmentation, and lysis were observed in most cells, sarcomeres were unevenly distributed. Activation of fibroblasts is accompanied by the growth of collagen fibers in the interstitium. Thyroid hormones affect endothelial functions mediated by the thyroid hormone receptor (THR)-α1 and THR-β. Activation of THR-α1 increases coronary blood flow, reduces coronary resistance in mouse models, and increases nitric oxide production in endothelial and vascular smooth muscle cells. Activation of THR-β by thyroid hormones induces angiogenesis by initiating a mitogen-activated protein kinase pathway. Severe hypothyroidism can also cause pericardial effusion due to increased capillary permeability and reduced lymphatic drainage from the pericardial space [17]. Hypothyroidism can affect the contractility of the heart and disrupt the relaxation of the heart muscle [9].

Conclusion

1. Under conditions of HHCy, hyper- and hypothyroidism in the myocardium of laboratory rats there are ultrastructural changes in the nuclear, contractile and energy apparatus of cardiomyocytes with the development of adaptive-compensatory and destructive changes.

2. Under the combined effects of hyperthyroidism and HHCy, hyper- and hypothyroidism, deeper remodeling of the hemocapillary wall with damage to the ultrastructure of endothelial cells and basement membrane was found. Against the background of insufficient microhemocirculation in cardiomyocytes revealed profound degenerative changes in the nuclei, components of the contractile and energy apparatus of the sarcoplasm, manifested by contractile zones, delibering and lysis of myofilaments, mitochondrial hypertrophy and lysis of their cristae.

References


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Гормоны щитотелоїной железы имеют значительное влияние на функцию сердца как через геномные, так и негеномные эффекты. Дефицит или избыток тиреоидных гормонов приводит к глубоким изменениям в регуляции сердечной функции и сердечно-сосудистой гемодинамики. Сердце является основным органом-мешалкой для гормонов щитотелоїной железы и у пациентов с гипо- и гипертиреозом происходят заметные изменения в работе сердца.

Целью работы было установление ультраструктурных изменений компонентов миокарда при условии экспериментальной гипергомоцистеинемии (ГГЦ) на фоне гипер- и гипотиреоза. Тиолактоновую ГГЦ моделировали введением телям эндогенного гомоцистеина (ГЦ) в дозе 100 мг/кг массы тела один раз на догое впродолжительности 28 дней. Гипертиреоз моделировали путем ежедневного введения L-тироксина в дозе 200 мкг/кг впродольше 21 дня, гипо-тиреозе - ежедневного введения мерказолила в дозе 20 мкг/кг массы впродольше 21 дня. Окремым групам телям вводили L-тироксин и мерказолил параллельно с ГЦ.

Высокие уровни ГЦ негативно влияли на стенки кровеносных сосудов миокарда. Просветы гемокапилляров были повреждены, заполнены эритроцитами. Влияние изменения в эндотелиоцитах, а кардиомиоциты миокарда деформированы. У лабораторных телям с гипертиреозом вставлялись нарушения ультраструктурных изменений в стенках кровеносных сосудов (наблюдается повреждение эндотелия, похолодение клеток миоцишных). У телям, которым моделировали гипертиреоз и ГГЦ, выявлены более значительные изменения в структуре миоцишных. Электронномикроскопическое исследование показало значительные ультраструктуры в стенках кровеносных сосудов, включая также вены и венозные вены миоцишных. Основной эффект гипертрофии сердца и ГГЦ выявлял наименьшую гибридные ультраструктуры кардиомиоцитов и гемокапилляров пороварено с ячейками гемокапилляров телям. Целостность и межклеточный контакт (включающая гибридные) миоцишных миоцишных миоцишных миоцишных миоцишных миоцишных миоцишных миоцишных миоцишных миоцишных миоцишных миоцишных миоцишных миоцишных миоцишных миоцишных миоцишных миоцишных миоцишных

Ключевые слова: гипертрофия, гипотрофия, гипергомоцистеинемия, миокард.