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MORPHOLOGICAL AND LECTIN HISTOCHEMICAL FEATURES OF WHITE NONLINEAR RATS MYOCARDIUM DURING EXPERIMENTAL HYPOTHYROIDISM

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ABSTRACT

General morphology and glycoconjugates of myocardium structural components has been studied in control and experimental animals. Experiments were performed on mature rats - males with weight 150-180g, who were divided into two groups (10 control and 10 experimental). Exogenous hypothyroidism was induced by daily administration of food thyreostatic drug mercazolilum at a dose of 7 mg / kg body weight within 30 days. After 30 days the animal euthanasia was performed by an overdose of ether anesthesia. Histological material (pieces of myocardium from ventricular areas and thyroid gland) were fixed in 4% neutral formalin, dehydrated, compacted and embedded in paraffin. Sections 5-7 μm thick were stained by hematoxylin and eosin for general morphology. Glycoconjugate carbohydrate determinants of myocardium structural components were studied by lectin-peroxidase technique using LABA (LFuc), HPA (NAcDGal), WGA (DGlcNAc>NeuNAc) lectins. Function control of thyroid gland was performed by investigation of thyroid glands morphology after staining slides by hematoxylin and eosin, and by determining T3 and T4 hormones in blood serum using radiological method by means of standard sets. It is shown that in hypothyroidism hypechromia of cardiomyocytes' nuclei, expansion of myocardial vascular lumen, perivascular edema and insignificant interstitial infiltration by leukocytes, local area accumulations of adipocytes are observed. Based on lectin histochemical studies it is shown modification of lectin receptors in the nuclei of cardiomyocytes and in endothelial cells of the microcirculatory bed, thus indicating the change of intensity of metabolic processes in cardiac myocytes and endothelial cells of the microcirculatory bed on the background of experimental hypothyroidism. DGlcNAc> NeuNAc-specific WGA lectin may be a marker of endothelial cells.

Keywords

Experimental hypothyroidism, myocardium, general morphology, lectin histochemistry.

Academic Discipline And Sub-Disciplines

Medicine, Histology

SUBJECT CLASSIFICATION

Pathological anatomy and histology

TYPE (METHOD/APPROACH)

Laboratory experiment, histochemical techniques, microscopic analysis

INTRODUCTION

Hypothyroidism is the clinical syndrome, caused by long lasting and persistent insufficiency of thyroid hormones in the body or by decrease of their biological effect on the tissue level. The main reason for the affection of the majority of organs in hypothyroidism is a sharp decrease in the synthesis of a number of enzymes due to deficiency of thyroid hormones [1, 2, 3, 4]. Violation in exchange of glycosaminoglycans leads to infiltration of the mucous membranes, skin, subcutaneous tissue, the myocardium. On the other hand violations of water-salt exchange is increased by an excess of vasopressin and natriuretic factor deficiency [1]. A special role is played by processes of accumulation of products of protein metabolism – glycosaminoglycans, derivatives of proteins, glucuronic and chondroitinsulfuric acids. They accumulate in the intersticium, causing mucoid (mucinous) edema [5]. Due to the high hydrophilicity of glycosaminoglycans water and sodium are accumulated in extravascular space: in the skin, heart, muscles, body cavities. Development of hydrothorax, hydropericardium, ascites is possible [6]. Hypothyroidism causes also changes in the myocardium, resulting in myocytes and interstitial edema, which ultimately is implemented in fibrosis [7]. Atrioventricular conduction and conduction in myocardium are disrupted. Violations of rhythm and conduction are diagnosed, which very ofter are resistant to the standard antiarrhythmic treatment. Heart dimensions are increased, its borders are shifted [8, 9, 3]. Abovementioned changes are the consequence of changes in myocardium, and consequence of mucinous effusion into pericardium with

formation of hydropericardium. The presence of peripheral edemas is regarded as a manifestation of heart failure. Although the edema may result from restrictive changes in myocardium and peripheral mucinous edema. In the myocardium during hypothyroidism there is significant edema of muscle fibers and interstitial tissue [7, 10, 11]. These changes are diffuse in nature, during long lasting hypothyroidism focal, and then diffuse fibrosis occurs. An objective study reveales an increase in heart dimensions, expanding of its borders. Increase in heart dimensions, edema of cardiac muscle cells and fibrous changes lead to violation of atrioventricular conduction [5, 6, 3].

It is known, that carbohydrate residues, which are part of cell glycoproteins, play the key role in the processes of morphogenesis, providing intercellular and cell-matrix interactions [12]. Changes in carbohydrate repertoire of cell membrane may lead to irreversible consequences in embryogenesis, development of lysosomal diseases or malignization in postnatal period. Analysis of dynamics of expression of lectin receptors on cell membranes allows you to answer about the level of functional activity of cells, ability for migration, phagocytosis, beginning of irreversible changes and apoptosis [13]. In the scientific literature available to us there are few data about the role of glycoconjugates in the functional activity of cardiomyocytes in norm and under conditions of thyroid hypothyroidism.

AIM

The aim of the work was to investigate the general morphology and carbohydrate determinants of glycoconjugates of myocardium structural components in control animals and during experimental hypothyroidism.

MATERIALS AND METHODS

Experiments were performed on mature rats - males with weight 150-180g, who were divided into two groups (10 control and 10 experimental). Exogenous hypothyroidism was induced by daily administration of food thyreostatic drug mercazolilum at a dose of 7 mg / kg body weight within 30 days. After 30 days the animal euthanasia was performed by an overdose of ether anesthesia. Investigations were carried out in accordance with the basic standards GCP (1996), the European Convention on Human Rights and Biomedicine from 04.04.1997, the Helsinki Declaration of the World Medical Association with the ethical principles of scientific medical research involving humans (1964-2008), the Order of the Ministry of Health of Ukraine № 690 from 23.09.2009 and in agreement with the Commission on Bioethics of Danylo Halytsky Lviv National Medical University (Protocol № 6 of 24.06.2013). Histological material (pieces of myocardium from ventricular areas and thyroid gland) were fixed in 4% neutral formalin, dehydrated, compacted and embedded in paraffin. Sections 5-7 μm thick were stained by hematoxylin and eosin for general morphology. Glycoconjugate carbohydrate determinants of myocardium structural components were studied by lectin-peroxidase technique using LABA (LFuc), HPA (NAcDGal), WGA (DGlcNAc>NeuNAc) lectins. Lectin receptors visualization was conducted in 3'3-diaminobenzidine tetrahydrochloride system in H_2O_2 presence. For specificity control of histochemical reactions were used: 1) lectin-peroxidase conjugates exclusion from staining protocol; 2) before lectin solution application, for oxidation of glycopolymers carbohydrate determinants, pre-incubation of histological slides was conducted during 60 min. in 1% HIO4 (Reanal, Budapest, Hungary). In the 1st case the results of histochemical reaction were completely negative, in the 2nd – essentially reduced. In addition to the above, as a kind of control of reaction specificity, negative staining of certain cellular compartments served on the background of lectin-reactive structures. Function control of thyroid gland was performed by investigation of thyroid glands morphology after staining slides by hematoxylin and eosin, and by determining T3 and T4 hormones in blood serum using radiological method by means of standard sets. Slides photographing was conducted, using a microscope "Granum R6053", equipped with camera "Echoo-Imager 502000", using computer program "ToupView 3.7". Morphometric investigations of thyroid glands parameters were performed by using computer program ImageTool for Windows (version 2.00), followed by statistical processing results using the MS Excel (Windows 2003). Results considered reliable at $p<0,05$.

RESULTS AND DISCUSSION

In rats of experimental group macroscopically thyroid glands increased in 2-3 times compared with the control group, microscopically - thyroid follicles acquired irregularly folded form, did'nt contain colloid or it was present in small quantities, cuboidal thyroid epithelium of the control group (Fig. 1 A) acquired cylindrical shape in experiment (Fig. 1 B), hyperplasia of thyroid cells and sharp organ hyperemia were detected. Similar results of thyroid gland morphology in the norm and during experimental hypothyroidism, induced by administration of mercazolilum (5 mg/kg), were observed by [14].

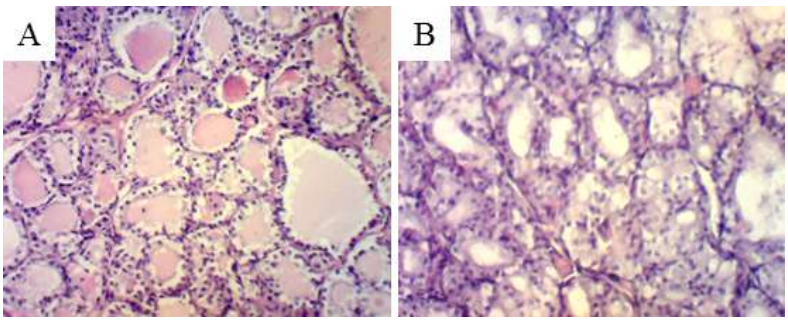

Fig 1: Thyroid gland of control and experimental rats. Staining by hematoxylin and eosin x150.

A - control, B – experimental hypothyroidism

Besides, the height of thyroid cells changed also. So, the height of thyroid cells of the rats in the control group was within 9,31 ± 0,49 μm, while in experimental animals the height of these cells was equal to 13,29 ± 0,29 μm, p<0,001.

Hypothyroidism was accompanied by changes in the level of thyroid hormones in the blood serum of experimental rats (T3 from 1,16±0,11 nmol / L till 1,04±0,13 nmol / L, p<0,01; and T4 from 50,0±3,39 nmol / L till 40,67±3,6 nmol / L, p<0,01).

Microscopical investigation of the heart wall has shown that myocardium has the typical structure and is formed of cardiac muscle cells with oxyfilic cytoplasm with spindle shaped nuclei in the center with small amount of heterochromatin. Between cardiomyocytes connective tissue septa with cellular elements and big amount of blood capillaries are clearly seen. Larger vessels, such as arteries, filled with blood plasma and blood cells, are visible also (Figure 2 A, C). It should be noted, that in hypothyroidism hypechromia of cardiomyocytes' nuclei, expansion of myocardial vascular lumen, perivascular edema and insignificant interstitial infiltration by leukocytes, local area accumulations of adipocytes are observed (Figure 2 B, D, E, F). Edema of myocardial connective tissue and nuclei' hyperchromia during thyroidectomy have been observed by [15].

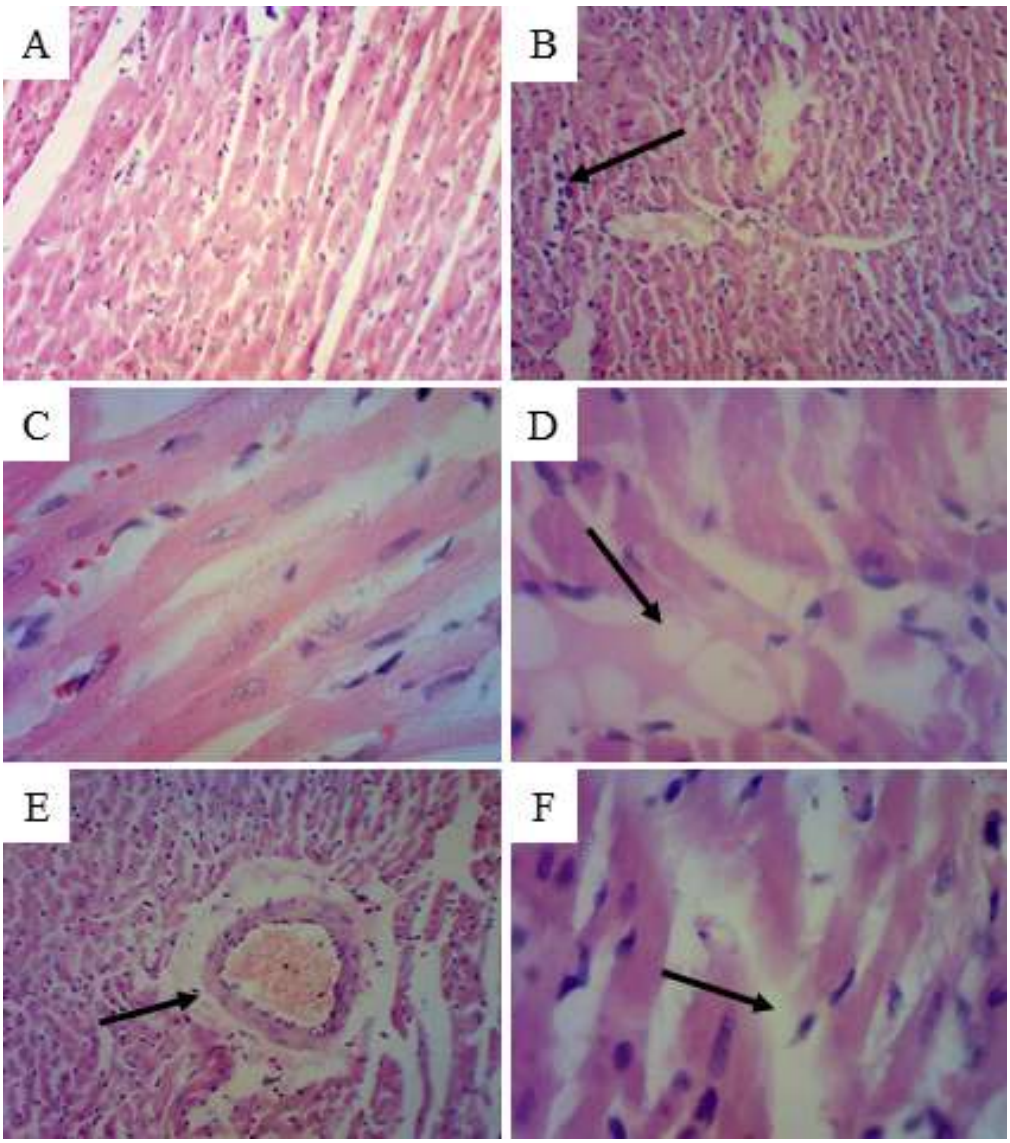

Fig 2: Overview slides of myocardium, staining by hematoxylin and eosin.

A – control, x150; C – control, x600;

B – (experiment), x150 – leukocyte infiltration (arrow);

D – (experiment), x600 – areas of adypocytes (arrow);

E – (experiment), x150 – perivascular edema around vessels (arrows);

F – (experiment), x600 – intersticial edema (arrow).

Lectin histochemical investigations showed the specificity of lectin binding with myocardial structural components (Table 1).

Table 1. The used lectins and their binding specificity intensity of lectins binding with structural components of heart wall

Name of lectin, its abbreviation, carbohydrate specificity*	Endothelium of myocardial vessels		Endothelium of myocardial vessels		Cardiac muscle cells		Vessels of epicardium	
	C	E	C	E	C	E	C	E
LABA (Laburnum anagyroides medik) Specific to LFuc	+ + +	+ + +	+	+ +	p.n.z + + +	c.e. +	+ +	+ + +
HPA (Helix pomatia l.) specific to NAcDGal	+	+	+	+	h.b.	p.n.z. + +	+ + +	e.n. + + +
WGA (Wheat germ agglutinin) specific to DGlcNAc>NeuNAc	+ + + b.m. + +	b.m. + + +	n. + + +	+ + +	p.n.z.. + + +	c.e. + + n.f. + + +	t.i. + +	

Note: + + + intensive binding; + + moderate binding, + weak binding, - no binding.

C - control, E – experiment (hypothyroidism).

n. – nuclei; e.n. – endothelial cells' nuclei; c. e. – cardiomyocytes' nuclei; b.m. – basal membrane; t.i. – tunica intima; h.b. – homogenic binding; n.f. – nerve fibers; p.n.z. – perinuclear zone.

*Carbohydrate specificity of the used lectins is given according to [12].

So, in myocardium of control animals WGA lectin receptors were associated with basal membrane of blood capillaries, endothelium, perinuclear zone of cardiomyocytes and with tunica intima of myocardial microcirculatory vessels (Figure 3). Expression of WGA lectin receptors were observed in endothelial cells of liver sinusoids [16] and in endothelium of skin blood capillaries [17]. Lectin HPA showed homogenous binding with myocardial structural components, but in the wall of epicardial vessels it has been shown considerably high its expression. Receptors of fucose-specific lecin LABA were exhibited in endothelium of vessels of microcirculatory bed and in perinuclear zone of cardiac muscle cells, and also in nuclei of endocardial endothelium and in nuclei of cells of subendothelial layer. During experimental hypothyroidism there were some differences in lectin binding, thus the moderate expression of HPA lectin receptors was declared in perinuclear zone of cardiac muscle cells and in nuclei of venous tunica intima. When compared with control group expression of WGA lectin receptors was higher in basal membrane of blood capillaries and in perinuclear zone of cardiomyocytes (Figure 3 B, D, F, H), also receptors of this lectin were detected in intersticial nerve fibers (Table 1). Fucose-specific lectin LABA retained high affinity to vascular endothelium (Table 1). Since thyroid hormones receptors are localized in nuclei, therefore on the background of hypothyroidism conformational changes of their structure are possible with the participation of carbohydrate component, namely DGlcNAc and LFuc, and influence on the synthesis of actin and myosin filaments and to change functional activity of cardiomyocytes.

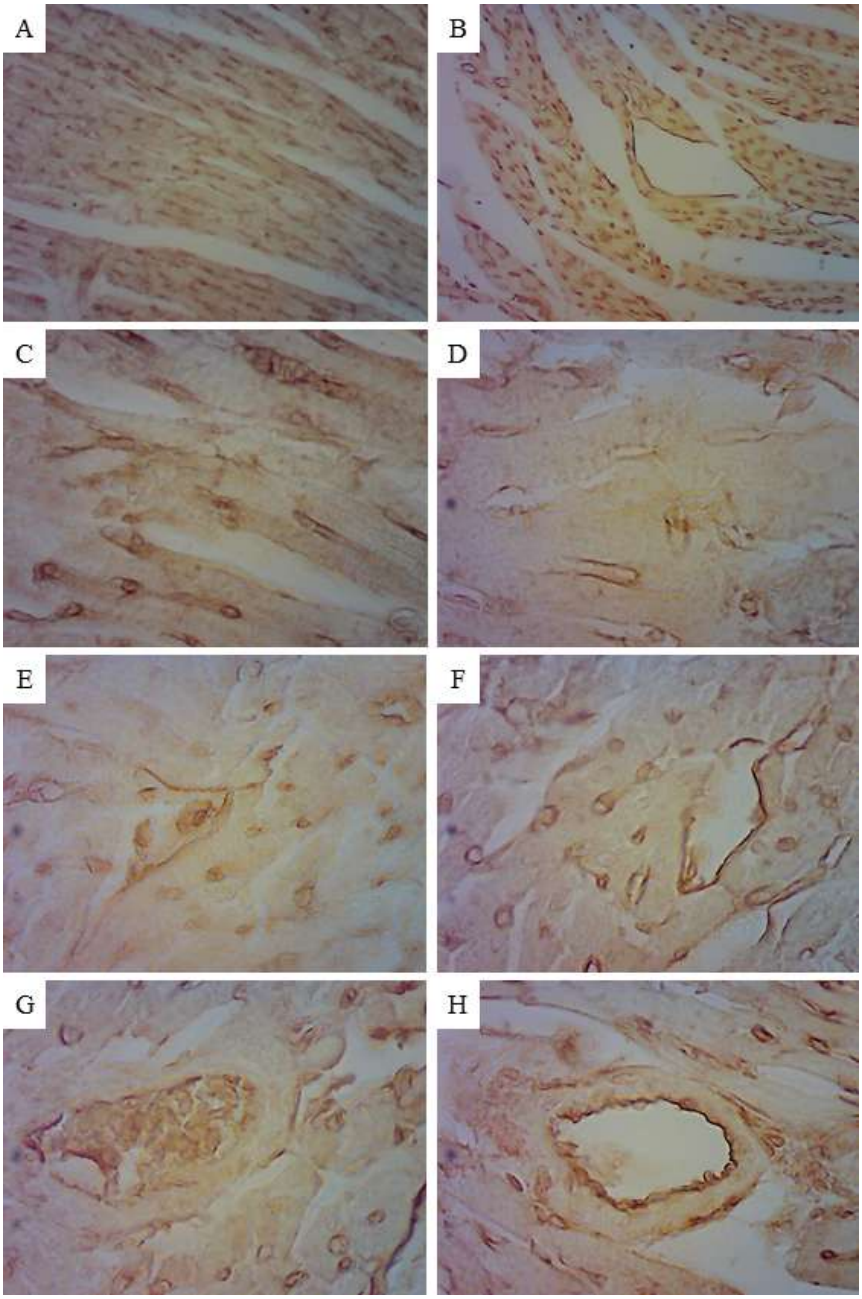

Fig 3: Expression of WGA lectin receptors in myocardial structural components.

A – control, x150; C, E, G – control, x600;

B – experiment, x150; D, F, H – experiment, x600

CONCLUSIONS

It is shown that in hypothyroidism hyperchromia of cardiomyocytes' nuclei, expansion of myocardial vascular lumen, perivascular edema and insignificant infiltration of interstitium by leukocytes, local area accumulations of adipocytes are observed. Based on lectin histochemical studies it is shown modification of lectin receptors in the nuclei of cardiomyocytes and in endothelial cells of the microcirculatory bed, thus indicating the change of intensity of metabolic processes in cardiac muscle cells and endothelial cells of the microcirculatory bed on the background of experimental hypothyroidism. DGlcNAc> NeuNAc-specific WGA lectin may be a marker of endothelial cells.

PERSPECTIVES FOR FURTHER RESEARCH

In the future it is planned to conduct ledtin histochemical research of the heart wall using a larger panel of lectins with different carbohydrate specificity on a background of hypo- and hyperfunction of the thyroid gland in the experiment.

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