ORIGINAL ARTICLE

SEX FEATURES OF THE DEVELOPMENT OF OXIDATIVE STRESS IN HIGH AND LOW HYPOXIC RESISTANCY RATS, WHICH EXPERIENCED REPEATED STRESSFUL EPISODES OF IMMOBILIZATION

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ABSTRACT

Aim: of the study was to find out the sexual characteristics of the development of oxidative stress in rats with high and low resistance to hypoxic hypoxia (HRH, LRH) during repeated stressful episodes of immobilization.

Materials and Methods: The study was performed on 96 white HRH, LRH male and female Wistar rats. The animals were divided into eight groups: 1 – control (HRH, LRH) males and females, 2 – immobilization (HRH, LRH) males and females. Immobilization stress was induced by gently restraining the animals four times by all paws and maxillary central incisors dorsally for 1 hour at an interval of 72 hours. The concentration of diene and triene conjugates (DC, TC), Schiff's bases (SB), TBA-active products (TBA-ap), activity of superoxide dismutase (SOD) and catalase (CAT), reduced glutathione (GSH), activity of glutathione peroxidase (GP) and glutathione reductase (GR) were determined in the homogenate of heart. A morphological study of heart preparations stained with hematoxilin and eosin was carried out. All studies were performed in control, after 24 hours after last immobilization.

Results: In control HRH, compared to LRH males, the values of TBA-ap were found to be lower. Under stress, an increase of LPO was noted in HRH and LRH males, only SB decreased. Higher level of LPO products noted in LRH rats compared to HRH. In control HRH females, compared to LRH, the content of DC, SB were higher and TBA-ap – lower. In HRH females, compared to HRH males, TC was higher and SB – lower. Under stress, in HRH females DC and TBA-ap were increased, TC and Sb – decreased. In LRH females all indicators increased. Lower TC and TBA-ap values were noted in HRH compared to LRH females rats. In control HRH males, compared to females, lower TC and higher values of SB were found. In LRH males, compared to females, the values of DC, TC, SB were higher. During stress, a more intensive course of LPO was noted in males.

SOD in control HRH males, compared to LRH, was higher; CAT was not statistically different. Under stress, SOD decreased, CAT – increased. In HRH males, compared to LRH, in this group SOD was higher CAT – lower. In the control, HRH females, compared to LRH, had higher SOD, lower – CAT. Under stress, CAT increased in HRH females; in LRH females CAT decreased and SOD – increased. In HRH females, compared to LRH, CAT was higher. In control males, compared to females, SOD and CAT was higher. Under stress higher SOD was in females, and CAT – in males.

GSH and GP were higher in control HRH compared to LRH males and females. Under stress, in males the GSH increased, GR decreased; GP in HRH decreased, in LRH it increased. HRH males, compared to LRH, GSH and GR were higher, GP – lower. Under stress, in females GSH, GP, GR in females decreased; HRH, compared to LRH, had higher GP and GH. In intact HRH males, compared to females, was higher GR and less GP; in LRH males, compared to females, GR was higher. Under stress, males had higher GSH, GP and GR values compared to females.

The studied biochemical changes in the heart are accompanied by alterative changes in the structural components of the myocardium in the experimental groups.

Conclusions: Congenital resistance to hypoxia is associated with a greater power of the enzymatic and non-enzymatic links of the antioxidant system. Immobilization stress repeated four times with an interval of 72 hours is accompanied by the most significant increase in the content of lipid peroxidation products in the heart homogenate of lower resistant to hypoxia rats, especially males. A decrease in Schiff bases is observed in all males and highly hypoxia-resistant females. In males, a more intense accumulation of lipid peroxide oxidation products, higher catalase activity and a more active glutathione system were noted. Females have significantly higher superoxide dismutase activity. Morphological changes confirm more damage to the heart of low hypoxia-resistant rats, more males.

KEY WORDS: heart, sex, rats, resistancy to hypoxia, biochemical changes, morphological changes, oxidative stress, lipid peroxidation, antioxydants

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INTRODUCTION

Stress is the most important protective and adaptive reaction that ensures the preservation of life in conditions of a changed environment. At the same time, the same stress can have different consequences for different people. For some, it is eustress and does not cause long-term and persistent disturbances in organs and systems and leads to adaptation, while for others it can be distress, which leads to disturbances [1, 2].

Under stress, the body adapts by help of maximizing the functions of the main systems, increasing metabolic processes and mobilizing all energy resources, which causes the development of energy deficit, tissue hypoxia and secondary organ dysfunction. However, in excessive or frequently repeated stress, adaptation is possible due to the reduction of metabolic processes, energy deficit, which leads to an increase in resistance to hypoxia [3]. High resistance to hypoxia is associated with a decrease

in aerobic and an increase in anaerobic glycolysis [4, 5], as well as a higher activity of antioxidants [6].

Under conditions of hypoxia, which occurs during stress, cell membranes are damaged due to the development of oxidative stress, as a result of hyperproduction of reactive forms of oxygen. With a lack of energy, the supply of oxygen increases due to hyperactivation of the cardiovascular system, which, together with the nervous system, suffers the most [7-9].

Hypoxia can trigger a large number of molecular and cellular processes that cause harmful changes in organs and the body as a whole. Thus, hypoxia underlies the development of most pathological processes, and the consequence of the disease depends on the body's response to it. It affects the severity of the course of such human diseases as coronary heart disease, stroke, infectious and non-infectious diseases, as well as the formation of multiple organ failure [10, 11].

It is known that in the general population of each biological species there are individuals with different resistance to lack of oxygen. The study of sexual characteristics of metabolic processes, which provide different mechanisms of adaptation to stress in animals with high and low resistance to stress, is an urgent task, the solution of which would help in choosing adequate correction methods.

AIM

To find out the sexual characteristics of the development of oxidative stress in rats with high and low resistance to hypoxic hypoxia (HRH, LRH) during repeated stressful episodes of immobilization.

MATERIALS AND METHODS

The work was doing at the Central Research Laboratory of I. Horbachevsky Ternopil National Medical University.

Morphological investigation of heart doing in Histology and Embriology of I. Horbachevsky Ternopil National Medical University.

All experiments were performed in the morning in a specially designated room at a temperature of 18-22 °C, relative humidity of 40-60% and illumination of 250 lux. Animals were kept and experiments on them in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes.

The research was carried out on 96 white sexually mature rats of different sexes, aged 5-6 months, weighing from 200 to 250 g, which were kept in one room on a standard diet and vivarium regime. All animals were divided into 2 experimental groups (Table 1).

Determination of resistance of animals to hypoxic hypoxia was carried out twice with an interval between tests of 14 days according to the method of V.Ya. Berezovsky, which is also used in modern research [12]. To determine the belonging of animals to one of the groups, we used the arithmetic mean survival value of all animals that were included in the experiment (M). The deviation was ±33% from M. Individuals whose survival time was longer than M+33% were classified as highly resistant to hypoxia (HRH), and individuals whose survival time was less than M-33% were classified as low-resistant to hypoxia (LRH). For experiments, animals were taken that twice confirmed belonging to one of the groups. In all studies, indicators were compared between HRH and LRH animals.

The control group included animals that were in standard vivarium conditions on a regular diet.

Immobilization stress was induced by gently restraining the animals four times by all paws and maxillary central incisors dorsally for 1 hour at an interval of 72 hours. A biochemical study of the heart homogenate of these animals was performed 24 hours after the last fixation [12].

Euthanasia of rats was performed by total bleeding from the heart after previous thiopental-sodium anesthesia (60 mg/kg-1 of the animal's body weight intraperitoneally).

In the heart of animals, the concentration of diene and triene conjugates (DC, TC), TBA-active products (TBA-ap), Schiff's bases (SB); superoxide dismutase activity (SOD) and catalase activity were determined.

The concentration of DC, TC and SB was determined according to the method [11], which is based on the fact that hydroperoxides extracted with a heptane-isopropyl mixture have a certain absorption maximum for DC at $\lambda = 232$ nm, for TC – at $\lambda = 278$ nm, for SB – at $\lambda = 400$ nm. The content of diene and triene conjugates, Schiff bases was expressed in units/g. TBA-ap was determined at a wavelength of 535 nm according to the method [13], expressed in micromoles per kilogram (μmol/kg).

Superoxide dismutase activity (SOD) in heart homogenate was determined according to the method [13], expressed in conventional units/g. Catalase activity (CAT) in heart homogenate and blood serum was determined according to the method [11], expressed in mcat/kg.

The concentration of reduced glutathione (GSH) was determined according to the method [14] and expressed

Table 1. Design of experiment – division of experimental animals

Group number	Investigation condition	Quantity of animal			
		Male		Female	
		HRH	LRH	HRH	LRH
I	Control	12	12	12	12
II	Immobilisation stress	12	12	12	12
Total		48		48	

in mmol/g. The activity of glutathione peroxidase (GP) and glutathione reductase (GR) in the heart homogenate was determined according to the method [13] and expressed in mmol/(min·kg).

Transverse sections of the heart at the level of both ventricles were taken immediately after decapitation of the animal and fixed in a 10% solution of neutral formalin. No sooner than two weeks later, the preparations were washed in running tap water and carried out in alcohol, poured into paraffin blocks. Sections were stained with hematoxylin-eosin and viewed under a light microscope [15].

Statistical processing of digital data was carried out using the software «Exel» («Microsoft», USA) and «STATISTICA» 7.0 («Statsoft», USA) using parametric and non-parametric methods of evaluating the obtained data. For all indicators, the value of the arithmetic mean of the sample (M), its variance and error of the mean (m) was calculated. The reliability of the difference in values between independent quantitative values was determined in the case of a normal distribution according to the Student's test, in other cases - using the U test of Mann-Whitney.

RESULTS

Changes in indicators of lipid peroxidation were detected (Table 2). In control HRH males, compared to LRH, the values of TBA-ap were found to be significantly lower by 7.4% ($p<0.001$).

Under stress, a significant increase in the primary and intermediate products of LPO was noted in HRH males: DC increased by 2.3 times ($p<0.001$), TC – by 3.6 times ($p<0.001$), TBA-ap – by 4.5 times ($p<0.001$), only SB decreased by 32.7% ($p<0.001$). In LRH males, the value of DC increased by 6.1 times ($p<0.001$), TC – by 6.9 times ($p<0.001$), TBA-ap – by 5.9 times ($p<0.001$), and SB decreased by 21.6% ($p<0.001$). Higher level of LPO products were noted in LRH rats compared to HRH. In particular, DC were 2.7 times ($p<0.001$) higher, TC – 2.0 times ($p<0.001$), TBA-ap – 1.4 times ($p<0.001$), SB – by 11,6% ($p<0.001$).

In control HRH females, compared to LRH, the content of primary and final products of lipid peroxidation was higher (DC – by 10.8%, $p<0.001$; SB – by 15.5%, $p<0.001$) and lower by 8.1 % ($p<0.001$) concentration of TBA-ap was determine). In HRH females, compared to HRH males, TC indicators were 15.0% ($p<0.001$) higher and 22.4% ($p<0.001$) was lower SB values.

Under stress, significant changes in LPO products were noted in HRH females: DC increased by 7.6% ($p<0.01$), TC decreased by 15.0% ($p<0.001$), TBA-ap increased by 47.5% ($p<0.001$) and SB decreased by 5.3% ($p<0.001$). In LRH females, all indicators increased: DC – by 20.4% ($p<0.001$), TC – by 12.0% ($p<0.001$), TBA-ap – by 94.2% ($p<0.001$), and SB – by 12.3% ($p<0.001$). Lower TC values were noted – by 11.3% ($p<0.001$) and TBA-ap – by 42.4% ($p<0.001$) in HRH compared to LRH females rats.

In control HRH males, compared to HRH females, 15.0% ($p<0.001$) lower TC indicators and 22.4% ($p<0.001$) higher values of SB were found. In LRH males, compared to LRH females, the values of DC were higher by 14.2% ($p<0.001$), TC by 6.8% ($p<0.05$) and SB – by 31.5% ($p<0.001$).

During stress, a more intensive course of LPO was noted in males: in HRH males, compared to HRH females, DC were 2.2 times higher ($p<0.001$), TC – 3.7 times ($p<0.001$), TBA-ap – by 3.1 times ($p<0.001$), and SB – by 9.2% ($p<0.001$). In LRH males, compared to LRH females, DC were 5.9 times greater ($p<0.001$), TC – 6.6 times ($p<0.001$), TBA-ap – 3.0 times ($p<0.001$).

Table 2. Changes in indicators of lipid peroxidation caused by immobilization stress in heart homogenate of high- and low-resistance to hypoxia rats of different sexes (M±m, n=12)

Group	Index			
	Diene conjugates, unit/g	Triene conjugates, unit./g	TBA-active products, μmol/kg	Shiff basese, unit/g
Higher resistancy to hypoxia males				
Control	0.957 ± 0.021	0.976 ± 0.013	0.892 ± 0.012	1.399 ± 0.030
Immobolisative stress	2,191 ± 0,057*	3,519 ± 0,033*	4,021 ± 0,036*	0,942 ± 0.019*
Lower resistancy to hypoxia males				
Control	0.983 ± 0.010	1.017 ± 0.019	0.953 ± 0.016**	1.340 ± 0.055
Immobolisative stress	6.013 ± 0.094*,**	7.027 ± 0.071*,**	5.618 ± 0.063*,**	1.051 ± 0.019*,**
Higher resistancy to hypoxia females				
Control	0.934 ± 0.024	1.122 ± 0.032$^{\#}$	0.885 ± 0.014	1.086 ± 0.016$^{\#}$
Immobolisative stress	1.005 ± 0.011$^{*,\#}$	0.954 ± 0.015$^{*,\#}$	1.305 ± 0.018$^{*,\#}$	1.029 ± 0.016$^{*,\#}$
Lower resistancy to hypoxia females				
Control	0.843 ± 0.016$^{**,\#}$	0.948 ± 0.017$^{\#}$	0.957 ± 0.015**	0.918 ± 0.016$^{**,\#}$
Immobolisative stress	1.015 ± 0.013$^{*,\#}$	1.062 ± 0.026$^{*,**,\#}$	1.858 ± 0.014$^{*,**,\#}$	1.031 ± 0.023*

*Note: * – indicators are reliable, compared to the control;*

*** – indicators are reliable, compared to HRH;*

 # – indicators are reliable, compared to males of the corresponding group.

Table 3. Stress-induced changes in the enzymatic activity of antioxidants in the heart homogenate of different sexes animals with high and low resistance to hypoxia (M±m, n=12)

Group	Index	
	Superoxide dismutase activity, units/g	Catalase activity, mcat/kg
Higher resistancy to hypoxia males		
Control	0.93 ± 0.02	1.57 ± 0.06
Immobolisative stress	$0.14 \pm 0.01^{*}$	$2.16 \pm 0.04^{*}$
Lower resistancy to hypoxia males		
Control	$0.69 \pm 0.01^{**}$	1.24 ± 0.06
Immobolisative stress	$0.06 \pm 0.02^{*,**}$	$2.56 \pm 0.04^{*,**}$
Higher resistancy to hypoxia females		
Control	$0.76 \pm 0.01^{\#}$	$0.30 \pm 0.03^{\#}$
Immobolisative stress	$0.74 \pm 0.02^{\#}$	$0.83 \pm 0.01^{*,\#}$
Lower resistancy to hypoxia females		
Control	$0.63 \pm 0.01^{**,\#}$	$1.07 \pm 0.01^{**,\#}$
Immobolisative stress	$0.79 \pm 0.02^{*,\#}$	$0.22 \pm 0.01^{*,**,\#}$

*Note: * – indicators are reliable, compared to the control;*
*** – indicators are reliable, compared to HRH;*
 # – indicators are reliable, compared to males of the corresponding group.

Changes of superoxide dismutase and catalase activities are presented in Table 3.

It was found that the SOD in control HRH males, compared to LRH, was higher by 34.8% (p<0.001). Catalase activity was not statistically significantly different in HRH and LRH animals. Under stress, SOD activity decreased in males: in HRH by 84.9% (p<0.001), in LRH – by 91.3% (p<0.001). Catalase activity, on the contrary, increased in HRH by 40% (p<0.001), in LRH – by 2.1 times (p<0.001). In HRH males, compared to LRH, in this group SOD activity was higher by 57.1% (p<0.001), catalase activity was lower by 18.5% (p<0.001).

In the control, HRH females, compared to LRH, had 20.6% (p<0.001) higher SOD activity, 3.6 times (p<0.001) lower catalase activity. Under stress, catalase activity increased 2.8 times (p<0.001) in HRH females. In LRH females, catalase activity decreased by 79.4% (p<0.001) and superoxide dismutase activity increased by 25.4% (p<0.001). In HRH females, compared to LRH, catalase activity was higher by 73.5% (p<0.001).

In control males, compared to females, superoxide dismutase activity was higher - in HRH by 22.4% (p<0.001), in LRH – by 9.5% (p<0.01) and catalase activity – in HRH by 80.9% (p<0.001), in LRH – by 13.7% (p<0.01). When comparing the results between males and females under stress, higher SOD activity was found in females: in HRH by 5.3 times (p<0.001), in LRH – by 13.2 times (p<0.001). In males, catalase values were higher: in HRH by 61.6% (p<0.001), in LRH by 91.4% (p<0.001).

The changes of the glutathione system, detected in experiment, are presented in Table 4.

GSH values were higher by 20.0% (p<0.001), GP – by 50.8% (p<0.001) in control HRH males compared to LRH males. The parameters of GR did not differ between the two groups of animals. Under stress, the value of GSH increased in males: in HRH by 1.8 times (p<0.001), in LRH – by 1.7 times (p<0.001). Also, GR activity decreased in HRH by 49.6% (p<0.001), in LRH – by 60.2% (p<0.001). GP activity in HRH decreased by 56.4% (p<0.001), in LRH it increased by 30.3% (p<0.001). Under stress, in HRH males, compared to LRH, the values of GSH were higher by 24.5% (p<0.001) and GR – by 22.1% (p<0.001), lower – GP by 47.1% (p<0.001).

In control HRH females, compared to LRH females, GSH values were higher by 22.0% (p<0.001) and GP by 57.5% (p<0.001). Such data are similar to the indicators in males, which indicates better antioxidant protection from the glutathione system in highly resistant to hypoxia rats. Under stress, the value of GSH in HRH females decreased by 41.2% (p<0.001), in LRH – by 30.6% (p<0.001). Also, the activity of GP decreased in HRH by 76.0% (p<0.001), in LRH – by 62.9% (p<0.001) and GR in HRH females – by 49.8% (p<0.001), in LRH – by 70.3% (p<0.001). Under stress, HRH females, compared to LRH, had higher values of GP by 34.2% (p<0.001) and GH by 44.8% (p<0.001).

When comparing the results between males and females, it was found that in intact HRH males, compared to HRH females, there was a higher value of GR by 53.2% (p<0.001) and less GP by 16.8% (p<0.001). In LRH males, compared to LRH females, only the activity of GR was higher by 55.7% (p<0.001). Under stress, males had higher GSH values compared to females: in HRH – by 65.6% (p<0.001), in LRH – by 58.1% (p<0.001). The activity of GP was also higher in males (in HRH by 35.8%, p<0.001, in LRH by 71.3%, p<0.001) and GR (in HRH by 53.4%, p<0.001, in LRH by 66.9%, p<0.001).

Table 4. Changes in the indicators of the glutathione system caused by stress in the heart of highly and low hypoxia-resistant animals of different sexes (M±m, n=12).

Group	Index		
	Reduced glutathione, mmol/g	Glutathione peroxidase activity, mmol/(min·kg)	Glutathione reductase activity, mmol/(min·kg)
Higher resistancy to hypoxia males			
Control	0.764 ± 0.022	0.429 ± 0.023	0.609 ± 0.024
Immobolisative stress	1.381 ± 0.039*	0.187 ± 0.024*	0.307 ± 0.022 *
Lower resistancy to hypoxia males			
Control	0.611 ± 0.025 **	0.211 ± 0.014 **	0.601 ± 0.034
Immobolisative stress	1.042 ± 0.016 *,**	0.275 ± 0.017 *,**	0.239 ± 0.021 *,**
Higher resistancy to hypoxia females			
Control	0.808 ± 0.013 #	0.501 ± 0.021 #	0.285 ± 0.019 #
Immobolisative stress	0.475 ± 0.021 *,#	0.120 ± 0.011 *, #	0.143 ± 0.010 *,#
Lower resistancy to hypoxia females			
Control	0.630 ± 0.012 **	0.213 ± 0.012 **	0.266 ± 0.021 #
Immobolisative stress	0.437 ± 0.017 *,#,**	0.079 ± 0.001 *,#,**	0.079 ± 0.001 *,#,**

*Note: * – indicators are reliable, compared to the control;*
*** – indicators are reliable, compared to HRH;*
 # – indicators are reliable, compared to males of the corresponding group.

The studied biochemical changes in the heart are accompanied by alterative changes in the structural components of the myocardium in the experimental groups.

When examining preparations of the myocardium of a HRH male rat after immobilization stress, the following changes were noted (Fig. 1). The lumens of the hemomicrocirculatory channel were filled with formed elements of blood. Contractile cardiomyocytes mainly kept their typical shape and location, nuclei were located mainly on the periphery of the cell, contoured in different ways against the background of unevenly illuminated sarcoplasm. Tincture heterogeneity of cardiomyocytes was observed in the myocardium, there were areas in the cells of which the nucleus was not visible, or it was shifted to the periphery. The fibers were swollen.

Morphological changes were the greatest in LRH males (Fig. 2). A significant expansion of the elements of

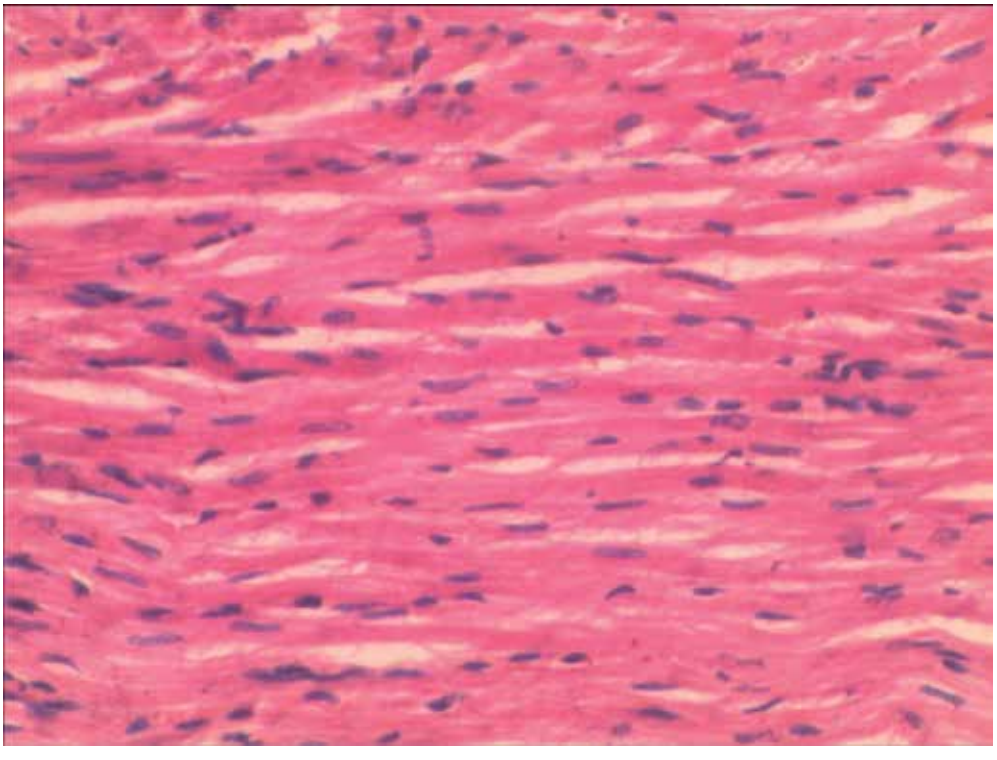

Fig. 1. A fragment of HRH male rat myocardium subjected to immobilization stress. Hematoxylin and eosin straining. x 200

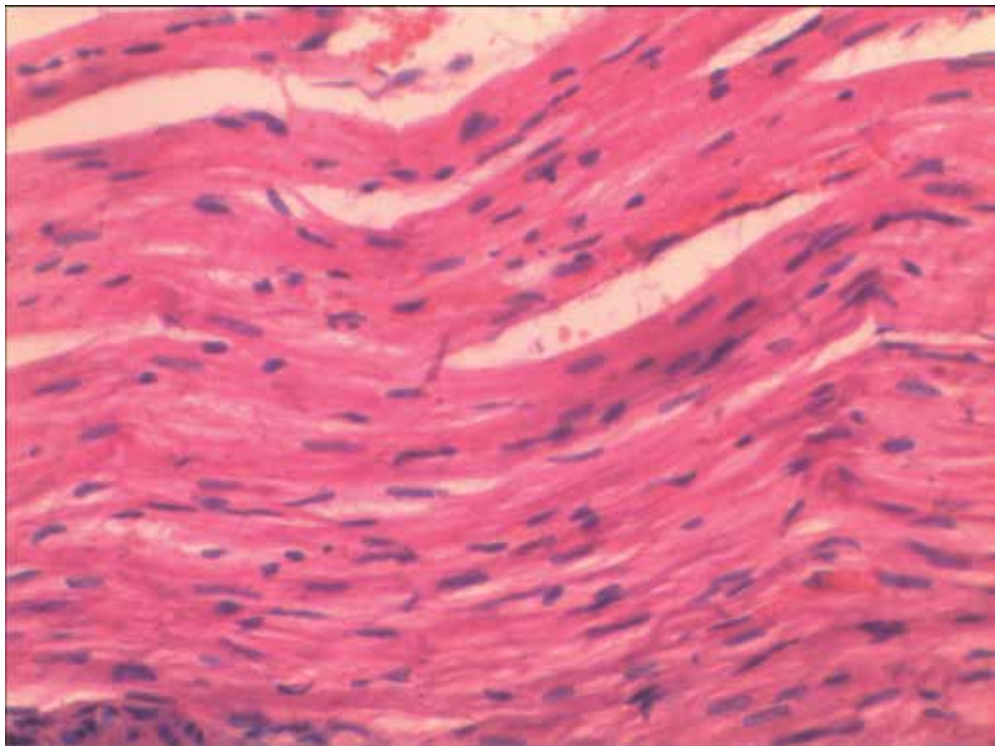

Fig. 2. A fragment of LRH male rat myocardium subjected to immobilization stress. Hematoxylin and eosin straining. x 200

the hemomicrocirculatory channel was noted, and their significant blood filling was accompanied by the exit of formed blood elements outside the vascular channel. Foci of lymphohistiocytic infiltration could be seen in the field of vision. Damage in the myocardium was characterized by small focal areas of altered cells. The coloring of the sarcoplasm of such cardiomyocytes was uneven due to its vacuolization. Against the background of the described changes, homogeneous areas of the myocardium with intense oxyphilic staining were also detected. Cell nuclei of such areas of the myocardium were not visualized. Violations of the tinctorial properties of the myocardium in LRH males were the most pronounced, myocardial tissue defibrillation was noted.

During the histological examination of the myocardium of the HRH of female rats subjected to stress (Fig. 3), it was found that its general structure was mostly preserved: the muscle fibers had an orderly arrangement, but the anastomoses between them were not well expressed everywhere. In the layers of the connective tissue between the fibers, the expansion of the elements of the hemomicrocirculatory channel was visible. A small amount of formed blood elements was observed in their lumens. The above-described areas of vasodilatation were focally located. Perivascular spaces did not differ from the norm. Contractile cardiomyocytes maintained their typical shape and arrangement. Tincture heterogeneity of cardiomyocytes was observed in some areas of the myocardium. Areas were also visible in the cells of which the nucleus was not visible. Some of the fibers were moderately swollen. Swelling of loose connective tissue was noted. Areas with leukocyte infiltration were visible in the field of vision.

In histological preparations of the hearts of LRH females that were subjected to immobilization stress, the tinctorial structure of the myocardium was more disturbed (Fig. 4). Layering of muscle fibers was noted. The animals had a large number of altered contractile cardiomyocytes, whose basophilic nuclei were dominated by heterochromatin. The nuclei of adjacent cells were disorganized in separate areas. The expansion of the components of the microcirculatory bed was more pronounced, compared to HRH, the lumens were filled with clusters of formed blood elements. The perivascular spaces were insignificantly expanded, somewhat illuminated, and lymphocytes were observed in them. Swelling of myofibrils and their homogenization were pronounced. Foci of leukocyte infiltration were noted.

DISCUSSION

In control HRH males, compared to LRH, the values of DC, TC and SB did not differ. Significantly lower values of TBA-ap were found only in HRH animals. In control HRH females, compared to LRH, there was a higher content of DC and SB, as well as a lower concentration of TBA-ap, and compared to HRH males – higher indicators of TC and lower values of SB. The obtained data indicate a more intensive course of LPO in intact HRH females, which may be related to a more powerful antioxidant system or the influence of estrogens. In LRH males, compared to LRH females, the values of DC, TC and SB were higher, which indicates a more intensive course of LPO in males.

The activity of superoxide dismutase was higher in the control groups of HRH males and females, compared to LRH of both sexes, which explains the lower values of

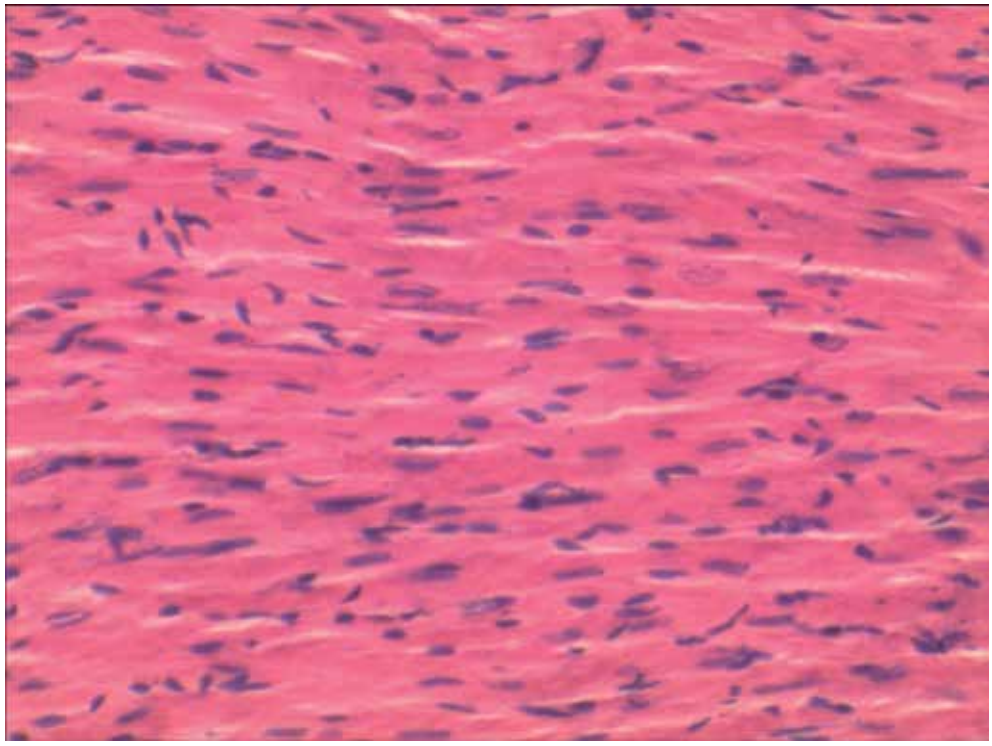

Fig. 3. A fragment of HRH female rat myocardium subjected to immobilization stress. Hematoxylin and eosin straining. x 200

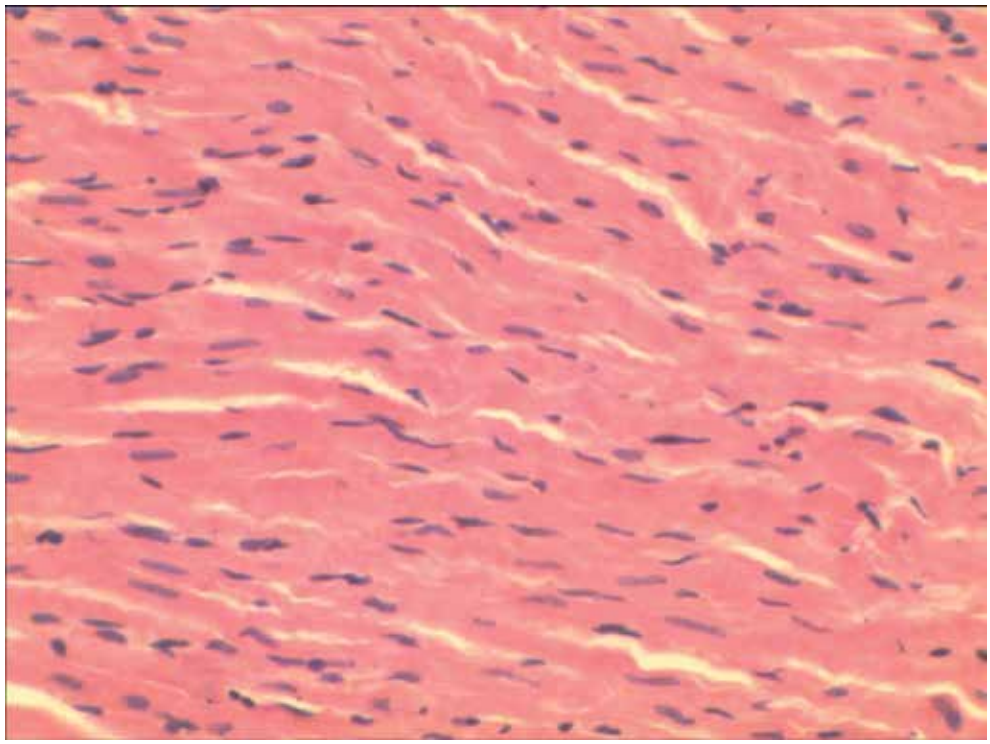

Fig. 4. A fragment of LRH female rat myocardium subjected to immobilization stress. Hematoxylin and eosin straining. x 200

the concentration of TBA-ap. Catalase activity was not statistically significantly different in HRH and LRH males, but among females it was higher in HRH. Such results indicate a greater power of the antioxidant system in HRH females, compared to LRH, during spontaneously occurring free radical processes. Superoxide dismutase and catalase activities were higher in control males (both HRH and LRH) compared to females. Given that the content of LPO products in males was also higher, one can think about increased metabolic processes in males, which may be related to sex hormones and greater activity in males.

When studying the glutathione system, higher values of GSH and GP were found in control HRH males and females, compared to LRH animals of both sexes, which indicates better antioxidant protection against oxidative processes in highly resistant to hypoxia rats.

During stress, a significant increase in LPO products: DC, TC, TBA-ap, as well as a decrease in SB, was noted in males. The detected changes indicate the accumulation of end products of peroxidation in rats under the influence of immobilization stress. In LRH males, significantly more accumulation of DC, TC and TBA-ap is noted, compared to HRH. In the latter, a more significant decrease in SB was noted. These changes may be associated with a lower capacity or higher expenditure of antioxidants in the LRH of males, as well as the absence of protective effects of sex hormones, particularly estrogens, on the heart. Other changes are observed in females under the influence of stress. Thus, an increase in DC and TBA-ap, as well as a decrease in TC and SB, was noted in HRH. At the same time, there is an increase in the concentration of all LPO products in LRH, and the values of TC and TBA-ap are higher in them than in HRH. These changes indicate a more pronounced stress in females with low resistance to hypoxia. When comparing the concentration of LPO products in the heart homogenate of rats of different sexes under the influence of immobilization stress, a more intensive course of biochemical processes was noted in males (both HRH and LRH, when compared with the corresponding groups of females).

In males under the influence of stress, regardless of resistance to hypoxia, the activity of SOD decreased and the activity of catalase increased. Changes in HRH of males are more pronounced, compared to LRH, which explains the lower concentration of LPO products in the homogenate of the heart of males with high resistance to hypoxia. As SOD activity significantly decreased, this led to pronounced nitrosative stress. True, catalase activity increased, which indirectly indicates the formation of H_2O_2. Perhaps superoxide dismutase worked to a greater extent precisely to prevent the formation of peroxynitrite, which led to its reduction in the heart. Changes in the SOD activity under stress were not observed in HRH females, but an increase in catalase activity was noted. At the same time, in LRH females, catalase activity significantly decreased, while SOD activity, on the contrary, increased. The obtained data indicate compensatory reactions that have different mechanisms of development depending on resistance to hypoxia. When comparing the results between males and females, significantly higher SOD activity was found in females and catalase activity in males. A lower activation of the enzyme link of the antioxidant system is observed in HRH individuals of both sexes, which may indicate their greater stress resistance.

In males, stress caused an increase in GSH value and a decrease in GH activity, regardless of resistance to hypoxia, but GH activity decreased in HRH males, and, on the contrary, increased in LRH. Higher values of GSH and GH, as well as lower values of GP, are observed in HRH males compared to LRH males. It is possible that the glutathione system is more actively involved in the detoxification of toxic substances during stress in HRH males. At the same time, a tolerant system of adaptation to stress is activated, which supports high resistance to hypoxia. In females, under the influence of stress, there is a decrease in the value of GSH, as well as a decrease in the activity of GP and GR, which indicates inhibition of the functioning of the glutathione system. At the same time, the activity of the mentioned enzymes is higher in HRH females than in LRH females. In males, compared to females, significantly higher activity indicators of the glutathione system were noted.

According to our previous results [16], in castrated rats, when adrenaline is injected, the increase in LPO and myocardial damage is significantly less, compared to non-operated animals, which indicates not only the protective effect of estrogens, but also the damaging effect of testosterone. The results of this work confirm this.

CONCLUSIONS

Congenital resistance to hypoxia is associated with a greater power of the enzymatic and non-enzymatic links of the antioxidant system. Immobilization stress repeated four times with an interval of 72 hours is accompanied by the most significant increase in the content of lipid peroxidation products in the heart homogenate of lower resistant to hypoxia rats, especially males. A decrease in Schiff bases is observed in all males and highly hypoxia-resistant females. In males, a more intense accumulation of lipid peroxide oxidation products, higher catalase activity and a more active glutathione system were noted. Females have significantly higher superoxide dismutase activity. Morphological changes confirm more damage to the heart of low hypoxia-resistant rats, more males.

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CONFLICT OF INTEREST
The Authors declare no conlict of interest

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