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CHANGES IN MALE REPRODUCTIVE SYSTEM UNDER ADVERSE ENVIRONMENTAL CONDITIONS

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ABSTRACT — The article investigates effects of adverse environmental factors under experimental conditions on different units of male reproductive system. White male rats weighing 210 g were exposed to microwave radiation of low intensity and natural gas containing hydrogen sulfide. The level of malonic dialdehyde (MDA) and kinetic indexes of lipoperoxidation were determined in homogenates of mediobasal hypothalamus and testes. Testosterone and lutein-izing hormone levels were determined in the blood serum by the enzyme immunoassay meth-od. Both microwave radiation and natural gas leads to a surge in the MDA level in testicular tissue and hypothalamus. The exposure to natural gas triggered the suppression of testicular steroidogenesis. The long-term exposure to natural gas caused profound biochemical changes in testicular tissue and epididymis by the level of protein. In general, the exposure to natural gas caused more serious changes in morphofunctional state of rat testes.

KEYWORDS — malonic dialdehyde (MDA), luteinizing hormone, testosterone, oxidative stress, lipoperoxidation, Leydig's cells, testicular tissue.

Oxidative stress is a state of imbalance between the systemic manifestation of reactive oxygen species and the ability of biological systems to detoxify readily the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Further, some reactive oxidative species act as cellular messengers in redox signalling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling.

In humans, oxidative stress is thought to be involved in the development of many diseases or may exacerbate their symptoms [10]. These include cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction, vitiligo, and chronic fatigue syndrome [5]. Oxidative stress also provokes infertility [1]. However, reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens [11]. Short-term oxidative stress may also be important in prevention of aging [3].

The stress concept considers stress to be physical and emotional [13]. At the same time stressing factors may be chemical agents (industrial toxicants, drugs) and food factors (disbalance in macro- and microelement content, deficiency in protein etc) [9]. In this connec-tion we have studied the effects of physical stress (microwave radiation) and chemical stress (natural gas containing hydrogen sulfide) in comparison, which will allow us to find out the ways of stress development as well as to disclose possible adaptive mechanisms within the male reproductive system.

The purpose of the work is to study the effects of adverse environmental factors under experimental conditions on different units of male reproductive system in comparison.

MATERIAL AND METHODS

The objects of the study were 80 white male rats weighing 200–220 g. The animals were subjected to the following stress conditions: 1) low intensity electromagnetic radiation; 2) natural gas containing hydrogen sulfide. The exposure with electromagnetic radiation was realized during 30 days for 30 minutes daily. To make electromagnetic field, the monochromatic electromagnetic wave generator was used («Yav-1-7.1», Russia; $\lambda = 7.1$ mm, frequency $f = 42.194$ GHz). To provoke chemical stress, the Astrakhanian natural gas was used. The natural gas exposure was carried out in concentration of 10 mg m^{-3} (by H_2S) during 30 and 120 days for 240 min daily. So, the following groups were formed: 1) Control group, 20 males; 2) experimental group 1 (E-1), 20 males (microwave radiation); 3) experimental group 2 (E-2), 20 males (gas exposure, 30 days); 4) experimental group 3 (E-3), 20 males (gas exposure, 120 days).

At the end of the experimental effects, the animals were decapitated under ether anesthesia in compliance with the Geneva Convention (1985). The level of malonic dialdehyde (MDA) and kinetic indexes of lipoperoxidation (spontaneous spLPO,

ascorbate-dependent ascLPO) were determined in homogenates of mediobasal hypothalamus and testes [12]. Peroxide resistance of erythrocytes was measured in blood [8]. Testosterone and luteinizing hormone (LH) levels were determined in the blood serum by enzyme immunoassay method. The protein content and fractional distribution of proteoglycans in epididymis and testes extracts were determined by electrophoresis method at pH 5 [7]. Sections of testicular tissue of 7 μm thick were made with the help of microtome "Microm HM-400" (Germany), and then stained with hematoxylineosin dye. The obtained preparations were studied using the universal microscope "Nu" (Germany) connected to the color television camera "Pixera" (USA). All the data obtained during the study were statistically processed using Student's criterion, the differences were considered significant at $p < 0.05$. The relationship between the studied param-eters was estimated by calculating the Pearson correlation coefficient (r) [4].

RESULTS

Microwave radiation (group E-1) caused an increase in erythrocyte peroxide hemolysis by 20%, compared with the control ($p < 0.05$), which indicates an increase in free radical oxidation in the blood and testifies to the development of oxidative stress. In group E-1, an increase in the dynamics of free radical oxidation processes was also observed in the testicular tissue: the initial level of MDA increased by almost 38.5 % compared to control values ($p < 0.001$). In the hypothalamic tissue, the initial level of MDA exceeded the control value slightly. At the same time, the kinetic indexes of lipid peroxidation, especially ascorbate-dependent, did not differ practically from the corresponding control values (Table 1). This fact indicates an insignificant effect of low-intensity microwave radiation on the regulatory unit of male reproductive system [2].

At the same time, in group E-1, the levels of testosterone and LH in blood plasma did not differ significantly from the control indexes, although it was found that the level of testosterone tended to decrease (Table 2). Thus, microwave radiation did not have a noticeable effect on the testosterone-producing activity of the testes, nor on the gonadotropic function of the adenohypophysis [2].

Under the influence of microwave radiation, a random arrangement of spermatogenic epithelial cells, or its uneven height, was observed. In some cases, multiple tears of the basal membrane were observed. An increase in the total number of Leydig cells at the expense of average size cells was also noted. Their area, however, tended to decrease. These contradictions explain the almost unchanged testosterone-producing activity of the testes under conditions of low-intensity microwave radiation (Fig. 1).

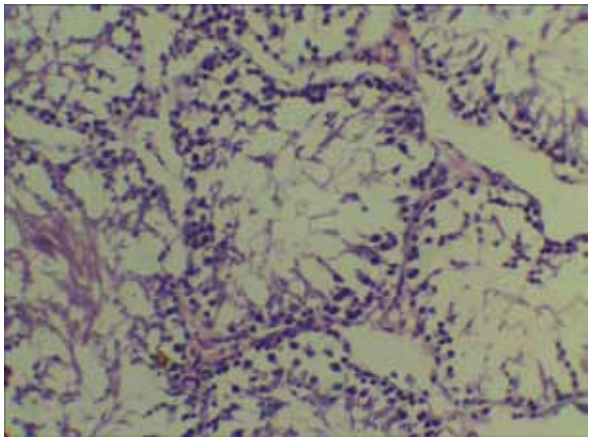

Fig. 1. *The structure of testicular tissue in rats exposed to microwave radiation. Stained by hematoxylin-eosin. Magnification 200 ×*

The exposure with natural gas during 30 days (group E-2) caused an increase in in erythrocyte peroxide hemolysis by 23.1 %, compared with the control group ($p < 0.05$) which testifies to the oxidative stress development. When exposed to hydrogen sulfide-containing gas, an increase in lipoperoxidation (LPO) processes in testicular tissue was observed. The initial level of MDA increased by 1.5 times compared to the control values. The kinetic in-dexes of LPO under conditions of gas intoxication increased significantly also, especially ascorbate-dependent lipid peroxidation. Therefore, under conditions of natural gas exposure, the intensification of radical oxidation processes takes place in testicular tissue, which results in functional state disorders in this tissue. In hypothalamic tissue, a significant increase in free radical processes was noted also, which indicates an inhibition of its functional activity in general. This, in its turn, negatively affects the functional state of adenohypophysis (Table 1).

The exposure to natural gas (group E-2) lead to a sharp decrease in the level of testosterone in blood plasma by 37.5% ($p < 0.001$) compared to the control indexes. The level of LH, however, did not change significantly, but tended to decrease. At the same time, the changes in testosterone and LH levels corresponded to a high coefficient of positive correlation $r = +0.808$ with a confidence of 95% (Table 2).

Under conditions of natural gas exposure the considerable decrease of diameters of seminal tubules and the increased quantity of interstitial tissue at the

Table 1. *Changes in lipoperoxide indexes in rat testes and hypothalamus under adverse conditions*

Experimental conditions	n	MDA, nmol/0,05 g	Kinetic indexes, nmol MDA h^{-1}	
			spLPO	ascLPO
Testicular tissue				
Control	20	4,89 ± 0,151	45,97 ± 0,840	48,74 ± 0,702
Group E-1	20	*** 6,77 ± 0,272	* 48,32 ± 2,003	*** 55,21 ± 0,894
Group E-2	20	*** 7,42 ± 0,457	*** 65,36 ± 3,104	*** 76,48 ± 2,431
Hypothalamic tissue				
Control	20	5,45 ± 0,280	56,68 ± 1,145	62,95 ± 1,451
Group E-1	20	*** 7,94 ± 0,506	59,28 ± 1,101	63,52 ± 2,007
Group E-2	20	*** 10,44 ± 0,398	*** 82,07 ± 1,023	*** 102,44 ± 4,013

** p < 0.05; ** p < 0.01; *** p < 0.001 compared with the control group*

Table 2. *Changes in testosterone and LH levels in blood plasma in rats*

Experimental conditions	n	Testosterone, ng cm^{-3}	LH, mMU cm^{-3}	r	p (r) <
Control	20	2,829 ± 0,0731	0,425 ± 0,0538	+0,935	0,05
Group E-1	20	2,614 ± 0,1160	0,420 ± 0,0068	+0,634	0,05
Group E-2	20	*** 1,769 ± 0,0814	0,370 ± 0,0152	+0,808	0,05

**** p < 0.001 compared with the control group*

expense of small Leydig's cells were observed. This may be considered as a compensatory reaction of endocrinocytes on the background of destructive changes in spermatogenic epithelium. At the same time the oedema of interstitial tissue and necrosis of spermatogenic cells took place. Seminal tubules were distantly located to each other. The chaotic distribution of spermatogenic epithelium cells and empty seminal tubules in a certain number of cases were observed. The height of spermatogenic epithelium was considerably decreased (Fig. 2).

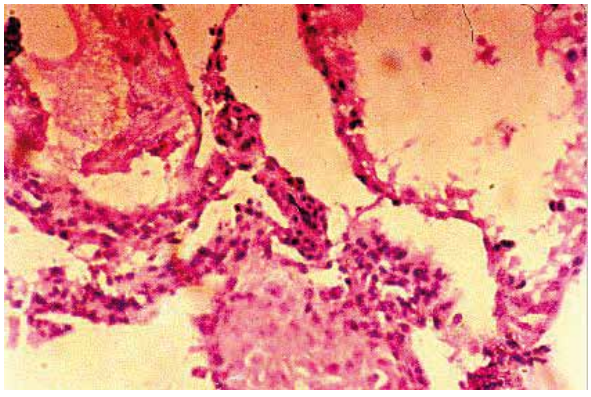

Fig. 2. *The structure of testicular tissue in rat exposed to natural gas. Stained by hematoxylin-eosin. Magnification 200 ×*

Therefore chronic intoxication with the natural gas containing hydrogen sulfide provokes testicular steroidogenesis depression that may be explained by radical formation processes as a result of oxidative stress development as well as endocrine changes in the system hypothalamus-pituitary [2].

In additional investigation (group E-3) we measured the average quantity of protein in proteoglycan samples from epididymis and testes. The average amount of protein in proteo-glycan samples in the control group was 9.5 ± 0.76 μg mg^{-1} (by the mass of the tissue). The exposure to natural gas during 30 days did not affect the concentration of protein significantly and the concentration seemed to be 9.8 ± 0.85 μg mg^{-1}. By the 120th day of exposure the amount of protein in proteoglycan fractions increased up to 7,4 ± 0,35 μg mg^{-1} which indicates a decrease in protein biosynthesis in testes. The amount of sulfates was 3.6 ± 0.3 μg cm^{-3} normally. The amount of sulfates in rats exposed to natural gas during 30 days did not differ from the control indexes. However, the level of sulfates began to increase only after long-term exposure, and by the 120th day of exposure to natural gas (group E-3) their level achieved to the value of 19.1 ± 0.85 μg cm^{-3}. This explains the increasing of the acidic proteoglycan fraction. Therefore, after three weeks' exposure with

natural gas profound biochemical changes occur in testicular tissue and epididymis.

CONCLUSIONS

Thus, the microwave radiation of low intensity provokes a damaging effect directly on spermatozoa, causing changes in the structure of spermatogenic epithelium. Under conditions of metabolic shifts caused by natural hydrogen sulfide-containing gas, reproductive dysfunctions are caused by both toxic effects of free radicals and inhibitory regulation from the hypothalamic-pituitary complex [2, 6]. The leading disturbances of morphofunctional state of testes under the influence of natural gas are spermatogenic epithelium necrosis, desquamation of germ spermatogenic cells in the lumen of the tubules, multiple ruptures of the basal membrane of seminiferous tubules on the the background of compensatory growth of interstitial tissue.

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