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STATE OF OXIDATIVE METABOLISM IN HEART AND SKELETAL MUSCLE TISSUES OF RATS UNDER CONTINUOUS LIGHTING

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The article considers and compares the features of oxidative metabolism of cardiac and skeletal muscle tissue under the changed photoperiod conditions, influenced by continuous lighting. It is shown that under the conditions of continuous lighting in the studied tissues there is an imbalance in the "prooxidants-antioxidants" system, a shift of balance towards peroxidation processes, which is manifested in increased generation of reactive oxygen species, increased concentration of peroxide oxidation secondary products, decreased activity of some antioxidant enzymes in the cardiac muscle tissue. In its turn, in the skeletal muscle tissue, a shift in balance is manifested in the decreased activity of some antioxidant enzymes.

Key words: prooxidant-antioxidant balance, lipid peroxidation, cardiac muscle tissue, skeletal muscle tissue, photoperiod.

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СТАН ОКИСНЮВАЛЬНОГО МЕТАБОЛІЗМУ В ТКАНИНАХ СЕРЦЕВОГО ТА СКЕЛЕТНИХ М'ЯЗІВ ЩУРІВ ЗА УМОВ ЦІЛОДОБОВОГО ОСВІТЛЕННЯ

У статті розглядаються та порівнюються особливості окиснювального метаболізму серцевої та скелетної м'язової тканини в умовах зміненого фотоперіоду, під впливом цілодобового освітлення. Показано, що за умов цілодобового освітлення у досліджуваних тканинах проявляється розбалансування у системі «прооксиданти-антиоксиданти», зсув балансу в бік процесів пероксидації, що проявляється у підвищенні генерації активних форм кисню, підвищенні концентрації вторинних продуктів пероксидного окиснення, зниженні активності деяких антиоксидантних ензимів у серцевій м'язовій тканині. В свою чергу, у скелетній м'язовій тканині зсув рівноваги проявляється у зниженні активності деяких антиоксидантних ензимів.

Ключові слова: прооксидантно-антиоксидантний баланс, пероксидне окиснення ліпідів, серцева м'язова тканина, скелетна м'язова тканина, фотоперіод.

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The conditions of modern reality, which are characterized by the pace of life acceleration, contribute to stressful situations. The stress response is formed due to the activation of the hypothalamic-pituitary-adrenal complex [9]. Oxidative stress is considered in the foreground of actively studied types of stress. It regulates its effects through the intensification of lipid peroxidation and the suppression of the antioxidant defense system [10]. Excessive activation of these processes is considered a key link in the pathogenesis of many diseases, which occurs due to stress factors [14]. Therefore, the mechanisms and consequences of the stress response in the body not only depend on the metabolic capacity of the body, but also on the biochemical nature of the damaging factors [4].

It is known that antioxidants can reduce the effects of aggressive free radicals, and one of the most powerful antioxidants is the pineal hormone melatonin. The active participation of the pineal gland's hormone melatonin at the present stage has shown the diversity of its physiological functions in the body, among which the most pronounced is antioxidant, immunomodulatory and stress-protective effect. Numerous studies have shown that the synthesis and secretion of the above hormone depends on light, and therefore the natural change in the regime of "light and darkness" is an important regulator of physiological functions of the body [2]. The modern rhythm of life significantly changes the light regime, as artificial lighting and lighting in the dark period of day have become an integral part of human life, accompanied by many serious health disorders [6, 11].

Despite the availability of scientific studies to study the effect of the altered photoperiod on the functioning of various organ systems, the issue of skeletal and cardiac muscle tissue's adaptation to these changes has not been sufficiently studied. Therefore, there is interest in studying the effect of altered lighting as a stress factor that can cause of skeletal and cardiac muscle function's impairment, as well as to compare how different types of muscle tissue respond to changes in the photoperiod.

The purpose of the study was to establish the features of the oxidative metabolism of cardiac and skeletal (for example, quadriceps femoris) muscle tissue under twenty-four-hour lighting.

Materials and methods. Experiments were performed on Wistar rats, with a mean weight of 220-260 g. Two groups of animals were formed. The first group consisted of 8 conditional animals (intact). The second group consisted of 8 animals, who have been kept under twenty-four-hour lighting for 30 days (intensity 1,000-1,500 lx). The use of this model is based on the fact that melatonin is synthesized by the pineal gland only in the dark, given that it is possible to assume that this model partially reproduces the lack of melatonin. It is known that 0.0005 mV/cm^2 of light power is enough for white rats to reduce melatonin production, but for some other rodents, a similar effect is caused by a light power greater than 1,850 mV/cm^2 [8].

Studies by some authors indicate suppression of pineal gland's functionality in mature male Wistar rats under twenty-four-hour lighting, which is accompanied by a decrease in the concentration of melatonin in the blood [12]. The works of L.O. Bondarenko and co-authors, GI Gubina-Vakulyk and co-authors [3, 5] showed that prolonged round-the-clock illumination causes the development of the pineal gland's hypofunction.

Experiments were performed in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986) and the Law of Ukraine "On the Protection of Animals from Cruelty" (2006)

The level of reactive oxygen species production was assessed by the formation of a superoxide anion radical according to reaction with nitrosine tetrazolium (Tsebrzhinsky OI, 2002). To assess the prooxidant-antioxidant system in the homogenate of the studied tissues the concentration of TBA-reactive products was determined (Stalnaya ID, 1977). The efficacy of the enzyme unit was assessed by catalase (Karolyuk MA, 1988) and superoxide dismutase (SOD) activity (Chevary S., 1985).

Results of the studies were analyzed by statistical data processing using the Shapiro-Wilk test, Student's t-test, Mann-Whitney U-test; calculations were performed using the "Microsoft Excel" software. The differences were considered statistically significant at $p<0.05$.

Results of the study and their discussion. Due to the influence of twenty-four-hour lighting on the oxidative metabolism status of cardiac and skeletal muscle tissue, the following results were obtained, which are shown in table 1.

Table 1

Biochemical parameters of rat cardiac and skeletal muscles oxidative metabolism under twenty-four-hour lighting conditions (M±m)

Index	Group	
	Intact group (n=8)	Twenty-foup-hour lighting (n=8)
Cardiac muscle		
·• O_2- mitochondrial electron transport chain (nmol .O_2-/g • sec) induced by NADH	12.734±0.675	15.665±0.724*, p<0.01
• O_2- microsomal electron transport chain (nmol .O_2-/g • sec) induced by NADPH	13.334±0.92	12.750±0.543
• O_2-phagocytes (nmol .O_2-/g • sec) induced by pyrogenal	1.244±0.044	1.666±0.012*. p<0.001
TBA-reactive products (μmol / kg)	61.80±4.50	96.50±0.79*. p<0.001
SOD activity (RU/g)	63.26±2.31	77.60±7.40*. p<0.001
Catalase activity (mcat/kg)	4.41±0.17	2.43±0.17*. p<0.001
Sceletal muscles (quadriceps muscle of thigh)		
• O_2- mitochondrial electron transport chain (nmol .O_2-/g • sec) induced by NADH	13.347±1.410	13.834±0.157
• O_2- microsomal electron transport chain (nmol .O_2-/g • sec) induced by NADPH	19.668±1.700	17.417±0.970
• O_2-phagocytes (nmol .O_2-/g • sec) induced by pyrogenal	3.265±0.190	3.111±0.160
TBA-reactive products (μmol/kg)	12.02±0.87	13.62±0.99
SOD activity (RU/g)	1.05±0.05	0.92±0.03*. p<0.05
Catalase activity (mcat/kg)	1.65±0.14	1.59±0.19

Note: * – the difference is reliable compared to the data of the intact group.

The presence of experimental animals in twenty-four-hour lighting did not affect the generation of superoxide anion radical in the tissue homogenate of the quadriceps femoris. But in the heart muscle, the production of the superoxide anion radical increased by 23 % due to the mitochondrial electron transport chain and by 34 % due to the activity of phagocytes. In this case, mitochondrial oxidation in the heart is the main supplier of energy due to aerobic β-oxidation of fatty acids and glucose. The load on this oxidation leads to greater production of superoxide anion radical. Because the superoxide anion radical damages the membranes, it is possible to suggest that a decrease in melatonin production by the pineal gland, by simulating twenty-four-hour lighting, leads to a decrease in antioxidant potential.

At the same time, damage of cardiomyocyte membranes by a superoxide anion radical of phagocytes is possible. It should be noted that an important role in the regulation of heart rate is played by the integrity of membranes and ion channels for Na +, K +, Ca + 2, Cl-. Thus, simulation of twenty-four-hour lighting can cause damage to cardiomyocytes by reactive oxygen species.

Analysis of the biochemical parameters of the prooxidant-antioxidant system showed that the concentration of TBA-active products in the heart muscle increased by 56 %, indicating increased processes of free radical peroxidation of biopolymers, which may be associated with both stressful light and with a lack of melatonin as an antioxidant. The concentration of TBA-active products in the homogenate of quadriceps femoris tissues did not change significantly.

The activity of antioxidant enzymes has undergone some changes, so the activity of superoxide dismutase in the homogenate of heart muscle tissue increased by 23 %, and the activity of catalase decreased by 45 %, which can be explained by reduced synthesis of melatonin, which stimulates the synthesis of this enzyme. Since catalase is a substrate-induced enzyme, and the substrate for it is hydrogen peroxide, it can be assumed that due to melatonin deficiency in the heart was increased production of hydrogen peroxide, which can be formed by activation of the prooxidant link.

The activity of antioxidant enzemes in the homogenate of skeletal muscle tissues of animals in the second group in comparison with animals of the intact group does not change significantly, except for superoxide dismutase activity. In animals of the experimental group, the activity of this enzyme is reduced by 12 %.

Analyzing the obtained results, it should be noted that an increase in the content of secondary peroxidation products in the homogenate of cardiac muscle tissue may indicate a weakening of antioxidant protection due to decreased melatonin secretion [13]. Indeed, under the conditions of the experiment, we observed almost no significant changes in the physiological parameters of the prooxidant-antioxidant system, which characterize the functioning of the heart and biochemical processes in it. In some ways, this can be seen as signs of similarity, nonspecific for such a short duration of the factor. It is known that the action of stressors of different origins causes the development of nonspecific adaptation syndrome, which consists in rats of three stages of different duration [7]. At the same time, the classic "anxiety reaction" occurs during the first two weeks, and specific signs of adaptation to the factor according to its origin are better manifested after almost two months. This pre-reaction of myocardial tissue may be due to the fact that almost all tissues, including the myocardium have receptors [15], both membrane and nuclear, to melatonin, and its excess, as well as the lack can directly affect the processes in cells.

It is known that ischemia also leads to increased lipid peroxidation with the formation of free radicals, which in its turn lead to damage of cardiomyocytes. Regulation of free radical oxidation processes takes place under the control of the enzymatic link of antioxidant protection. Thus, the increase in the concentration of secondary peroxidation products in the homogenate of cardiac muscle tissue indicates an increase in the level of free radical peroxidation of lipids, and is an indicator of reduced antioxidant potential in hypofunction of the pineal gland and may be associated with stress effect, and lack of light and deficiency of melatonin as an antioxidant, but in any case it may indicate a stress response development. Analyzing changes in catalase activity, it should be remembered that this enzyme is vital in the mechanisms of maintaining normal levels of hydrogen peroxide metabolism and prevents its accumulation in cells, reducing H_2O_2 to molecular oxygen and two water molecules. That is, it can be noted that compared to other reactive oxygen species, hydrogen peroxide is a quite stable compound with low reactivity. However, in contrast to O_2, hydrogen peroxide can penetrate cell membranes and react with cellular components quite distant from the site of synthesis. On the other hand, it is well known that an increase in H_2O_2 causes a number of reactions in the cell, such as an increase in intracellular concentration of Ca^{2+}, changes in the activity of Ca-ATPase, due to the oxidation of its sulfhydryl groups.

Increase in the activity of superoxide dismutase as the main producer (especially in the absence of catalase activity) of H_2O_2, which with a superoxide anion radical can give the OH radical by the Haber-Weiss reaction,it can contribute to arrhythmias.

Decreased superoxide dismutase activity in skeletal muscle tissue homogenate may indicate activation of peroxidation and weakening of the antioxidant link in this group, and may be a marker to identify the source of inflammation or oxidative destruction of the studied tissue due to exposure to a stress factor. These results coincide with the known data on the course of biochemical processes under the action of stress factors [5]. It can be assumed that a decrease in the activity of such an antioxidant enzyme as superoxide dismutase indicates the chronic nature of the stress factor, which is a change in the photoperiod (twenty-four hour lighting). The results of the prooxidant-antioxidant status study indicate a lack of power of the enzymatic link of antioxidant protection.

It is well known that an important role in ensuring the normal functioning of the body is played by the biological rhythms of the cardiovascular system. The main function of the cardiovascular system is transport of oxygen to the tissues, so the intensity of its functioning is determined by the level of energy metabolism in the tissues. In order to sustain life in an ever-changing environment, the body must respond to these changes. Thus, the body's response is to maintain homeostasis and "triggered" by regulatory systems. The heart rhythm can serve as an assessment of the regulatory systems not only of the heart but also of the body as a whole, because it is the cardiovascular system that first responds to any influence, therefore, the adaptive stress of balancing the body with the environment is due to the increase in the regulatory processes' strain.

It is established that the level of energy metabolism increases during the day and decreases at night. This is due to the activation of the sympathetic-adrenal system during the day, which provides oxygen transport by increasing cardiac output. The main regulator of circadian rhythms of the human body is the pineal hormone melatonin [1, 13]. Normally, at night, its production increases, which reduces the activity of the sympatho-adrenal system and the level of metabolism. Therefore, in recent years, more and more attention is paid to the search for new biological markers that could expand the possibilities of timely diagnosis and prediction of this pathology. Active participation of the pineal hormone melatonin at the present stage has shown the diversity of its physiological functions in the body. Impairment of the level and rhythm of melatonin secretion is the cause of a number of cardiovascular diseases [6].

Perhaps free radical peroxidation, as a non-enzymatic process, is a price to pay for organization of metabolism, which affects regulation, so changes in the prooxidant-antioxidant system may be components of the most common causes for mechanisms of adaptation such states as pineal gland's hypofunction.

We assume that changes in the prooxidant-antioxidant system of experimental tissues in the conditions of the pineal gland's hypofunction are not only indicators, but also factors that result in adaptations. However, such balance changes under the conditions of long-term hypofunction may be one of the factors disrupting the adaptive mechanisms in which the level of protective physiological mechanisms is reduced.

Thus, the experiments showed that keeping rats in a 30-day twenty-four-hour lighting enhanced the processes of peroxidation, the intensity of antioxidant protection and reduced its regenerative potential in the studied tissues.

Cjnclusions

1. The presence of experimental animals under twenty-four-hour lighting did not affect the generation of superoxide anion radical in the homogenate of the quadriceps femoris tissues. But in the heart muscle, the production of the superoxide anion radical increased by 23 % due to the mitochondrial electron transport chain and by 34 % due to the activity of phagocytes.

2. The 30-day exposure of rats under continuous light showed significant signs of increased peroxidation in the heart of rats, which was expressed in an increase in the concentration of TBA-active products by 56 % against weakening of antioxidant protection, manifested in a decrease in catalase activity by 45 %; the quadriceps femoris tissue also shows weakening of antioxidant protection by reducing superoxide dismutase activity by 12 %. These changes may indicate an imbalance in the prooxidant-antioxidant system of the studied tissues.

Prospects of further research. Based on the obtained results, we consider it expedient to continue the study of the influence of the altered photoperiod as a stress factor on the prooxidant-antioxidant balance of cardiac and skeletal muscle tissue, in particular to consider changes under light deprivation on the above structures.

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