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# THE ACTIVITY OF $Na^+$, $K^+$-ATPase AND ADENINE NUCLEOTIDES CONTENT IN THE HEART MUSCLE OF RATS UNDER CONDITIONS OF COMBINED EFFECT OF IONIZING RADIATION AND INTENSE PHYSICAL EXERCISE

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## Abstract

There is a high risk of radiation contamination of the environment due to warfare taking place on the territory of Ukraine. The impact of ionizing radiation on the body is often combined with heavy physical exertion, which significantly exacerbates its negative effects. Many systems of the living organism become compromised and in particular the cardiovascular system. Therefore, the aim of this article is to study the active ion transport systems functioning and the adenine nucleotides content in the mitochondrial fraction of the heart muscle from irradiated rats subjected to intense physical exercise.

Experimental studies were carried out on 50 sexually mature male Wistar rats. The animals were irradiated at a total dose of 154,8 mC/kg on a γ-therapeutic device AGAT-R No. 83 (isotope $^{60}Co$) under the following technical conditions: source-field distance 75 cm, dose rate 0,385 mA/kg. After 1 hour, 1 day, and 3 days after the exposure, the rats were subjected

to heavy physical exertion, which was simulated using a treadmill. When the animals refused to run, they were decapitated. In the mitochondrial fraction of cardiomyocytes, the activity of $Na^+,K^+$-ATPase, the content of adenine nucleotides, their ratio and adenylate energy charge were measured.

1 hour after total-body irradiation and intense physical exercise, the content of ATP and the activity of $Na^+,K^+$-ATPase decrease by 29% and 39%, respectively, compared to the control group. However, compared to the group of animals that underwent intense physical exertion only, these parameters remain relatively unchanged.

24 hours after the irradiation, there is a certain increase in the concentration of ATP and $Na^+,K^+$-ATPase enzyme activity in relation to the previous period by 24% and 13%, respectively. That indicates an activation of compensatory and adaptive mechanisms. Still, no complete normalization of energy levels and active ion transport through the cell membrane is observed.

The most prominent changes in the studied parameters are recorded on the 3rd day of the experiment, which coincides with the onset of acute radiation syndrome. There is a discharge of the adenylate energy system of the myocardial cells, which is evidenced by a significant decrease in the energy charge, ATP/ADP and ATP/AMP ratios. During this period, the activity of $Na^+$, $K^+$-ATPase is suppressed by 55% compared to the control group and by 19% in relation to non-irradiated exhausted animals.

The authors concluded that the effect of total-body gamma irradiation at a dose of 154,8 mCl/kg followed by intense physical exercise leads to a more significant suppression of $Na^+$, $K^+$-ATPase activity in the heart muscle of rats and to a disturbance in the adenylate energy system compared to the animals that underwent physical exertion only.

Negative changes in the studied indicators are determined by the time that has passed after the total-body gamma irradiation with a tendency to worsen on the 3rd day of the experiment.

**Key words: rats; ionizing radiation; physical exercise; heart muscle; $Na^+$; $K^+$; ATPase; adenine nucleotides.**

## Introduction

The consequences of the Chornobyl accident in 1986 have a major impact on the population and the environment. These issues become especially relevant in view of the military actions on the territory of Ukraine. A high risk remains that the aggressor country may use nuclear weapons or carry out terrorist attack at nuclear power plants, which in turn

increase the risk of radiation exposure of both civilians and military personnel [1]. Military service under the conditions of high stress level has similarities with works during elimination of the consequences of the Chornobyl disaster. There are quite a lot of scientific papers dedicated to the study of the cardiovascular system Chornobyl liquidators. Numerous changes in the structural and functional aspects of that system were discovered, which did not disappear even in remote periods after radiation exposure. The investigators noted higher prevalence of atherogenic types of dyslipoproteinemia, activation of free radical reactions and destabilization of cell membranes caused by suppression of energy metabolism, a decrease in the contractile capacity of the myocardium, the rapid development of hypertension and coronary heart disease, various changes in heart rhythm, conductivity, etc. [2, 3, 4, 5].

The impact of ionizing radiation on the body is often combined with heavy physical exertion, which in turn significantly increases its negative consequences due to the intensification of oxidative stress, dysfunction of active ion transport systems in cell membranes, ATP deficiency and energy depletion [6, 7, 8, 9].

**Purpose of the study**

This research aims to explore the functioning of the active ion transport systems and adenine nucleotides content in the mitochondrial fraction of the heart muscle of irradiated and physically exhausted rats.

**Materials and methods**

The research was conducted on 50 sexually mature male Wistar rats, divided into 5 groups of 10 animals each. Group 1 – intact (control group); group 2 - rats that were subjected to intense physical exercise only; group 3 - rats that underwent intense physical exercise 1 hour after radiation exposure; Group 4 - rats that underwent intense physical exercise 24 hours after radiation exposure; Group 5 - rats that underwent intense physical exercise 72 hours after radiation exposure;

Animals were subjected to total-body gamma irradiation of $^{60}$Co at a dose of 154.8 mC/kg using a gamma-therapeutic device AGAT-R No.83 under the following technical conditions: source-field distance 75 cm, dose rate 0.385 mA/kg. After 1 hour, 24 hours, and 72 hours after the exposure, the rats were exposed to heavy physical exertion using a treadmill. The length of a treadmill belt was 80 cm, at the end of which was a stimulating grid delivering electric shocks of 24V of alternating current each time the animal stopped running and touched the grid. The running belt speed was set at 0.5 m/s, the incline angle was 10°. The exhaustion was defined as a moment when the rat refused to run, no longer reacting to electrical stimuli.

Rats were killed by decapitation in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986). In the mitochondrial fraction of the heart muscle, the activity of $Na^+$, $K^+$-ATPase [10] and the content of adenine nucleotides were measured using Boehringer Test-Combination kits. Enzyme activity was expressed in micromoles of inorganic phosphate per mg of protein in the sample for 1 minute of incubation. The obtained data was statistically processed by comparing calculated t-score with the critical values from the t-table. The software used was "Primer Biostatistics" and "Excel".

**Discussion**

In the heart muscle (Fig. 1) intense physical exertion in the first hour after gamma irradiation led to a decrease in ATP levels by 29% compared to the control group, however in comparison with the group of animals that underwent only heavy physical exertion, no significant changes occurred.

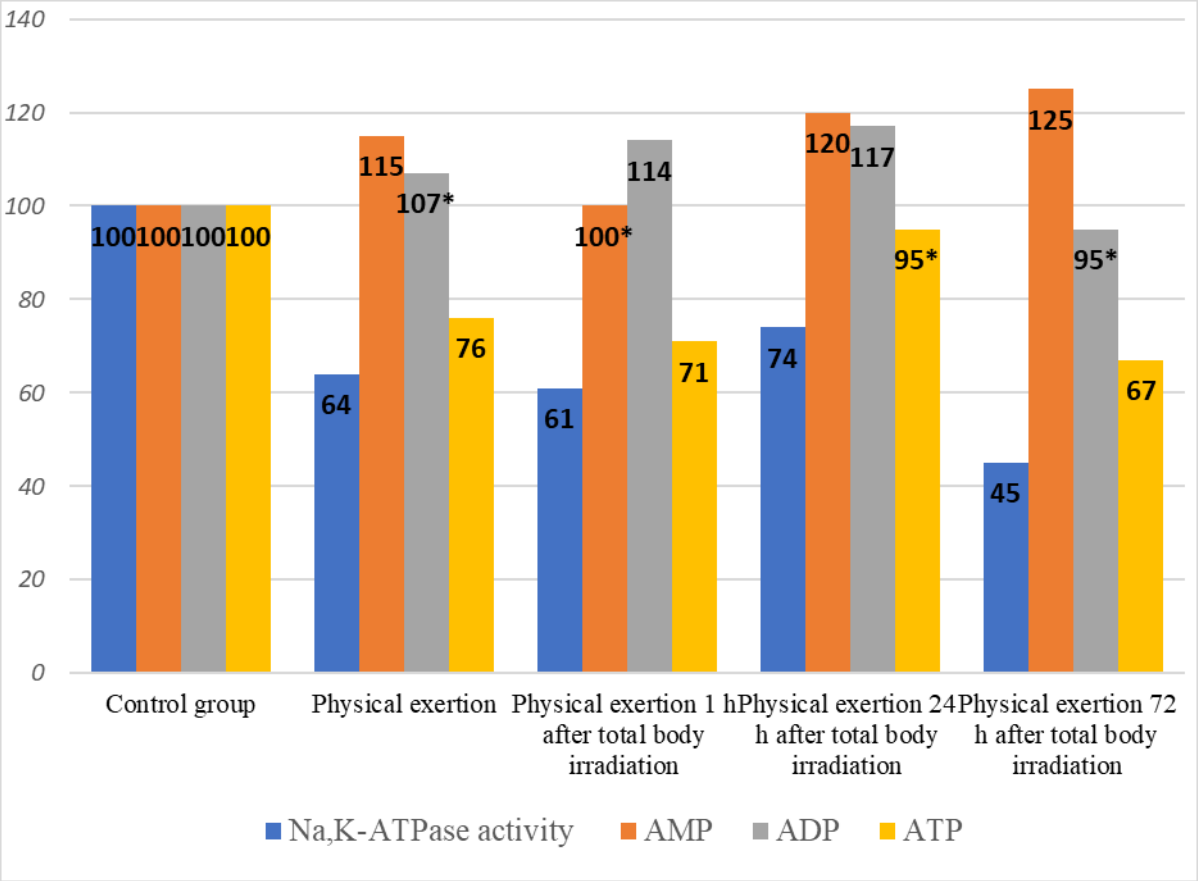


**Fig. 1. Activity of $Na^+$, $K^+$-ATPase (%) and content of adenine nucleotides (%) in the mitochondrial fraction of heart muscle cells in comparison with the parameters of the control group; *- p> 0.05.**

The content of ADP increased by 14% in relation to intact rats, remaining almost equal to ADP levels in non-irradiated exhausted animals. The level of AMP was similar to that in the control group, but compared to non-irradiated exhausted rats, it was 15% lower. In the same period of time, the activity of $Na^+$, $K^+$-ATPase decreased significantly by 39% compared to the control group and practically identical in comparison with animals that were subjected to physical exercise only.

24 hours after radiation exposure, exhaustive exercise led to a significant increase in ATP content, almost reaching control values. The content of ADP and AMP increased in comparison with the intact group by 17% and 20% respectively, and by 10% and 5% in relation to the animals that underwent physical exertion only. Energy charge value raised as well (table 1). This can be explained by activation of compensatory and adaptive mechanisms. Regardless, the complete normalization in the energy supply of myocardial cells did not occur, as evidenced by decreased ATP/ADP and ATP/AMP ratios, which are 79% and 77% of control level respectively. The activity of $Na^+$, $K^+$-ATPase in this group of rats increased by 13% compared to the previous group and by 10% in comparison with non-irradiated physically exhausted animals.

72 hours after irradiation, which coincides with an onset of acute radiation syndrome, exhaustive exercise caused a significant decrease in the ATP amounts by 33% compared to the control, and by 9% in relation to the group of animals that were subjected to heavy physical exercise only. During that period, the level of ADP in the myocardium decreased, almost reaching control levels. Nevertheless, the content of AMP continued to increase compared to the previous periods of the study. The described changes allow us to conclude that intense physical exertion 72 hours after gamma irradiation leads to a discharge of the adenylate energy system of myocardial cells, which is evidenced by a decrease in the energy charge and the ATP/ADP and ATP/AMP ratios. In this phase of acute radiation syndrome the activity of $Na^+$, $K^+$-ATPase is suppressed significantly by 55% compared to the activity in the intact group and by 19% in relation to non-irradiated exhausted animals.

Thus, the combined effect of ionizing radiation and exhaustive exertion on the body leads to more pronounced changes in functioning of $Na^+$, $K^+$-ATPase and adenylate energy system compared to physical exertion alone. During the analysis of the obtained results, it is also possible to note the time-dependent nature of fluctuations of cell energy supply parameters and active ion transport through biological membranes with a significant deterioration on the 3rd day of the experiment, which indicates a decrease in the compensatory abilities of the heart muscle cells during this period.

Table 1

**The content of adenine nucleotides in the mitochondrial fraction of rat cardiac muscle cells after exposure to γ-irradiation at a dose of 154,8 mC/kg and intense physical exercise**

| Nucleotides | Intact animals | Intense physical exercise | Onset of intense physical exercise after gamma irradiation exposure | | |
|---|---|---|---|---|---|
| | | | 1 hour | 24 hours | 72 hours |
| Amount of adenine nucleotides | 3,409 ± 0,200 | 2,813 ± 0,040<br>p< 0,01 | 2,730 ± 0,060<br>p< 0,01<br>p´> 0,05 | 3,382 ± 0,010<br>p> 0,05<br>p´< 0,001<br>p″< 0,001 | 2,599 ± 0,010<br>p< 0,001<br>p´< 0,001<br>p″< 0,001<br>p´″< 0,001 |
| Energy charge | 0,839 ± 0,010 | 0,784 ± 0,005<br>p< 0,001 | 0,784 ± 0,004<br>p< 0,001<br>p´> 0,05 | 0,807 ± 0,002<br>p< 0,01<br>p´< 0,001<br>p″< 0,001 | 0,768 ± 0,002<br>p< 0,001<br>p´< 0,01<br>p″< 0,001<br>p´″< 0,001 |
| ATP/ADP | 4,766 ± 0,500 | 3,254 ± 0,080<br>p< 0,01 | 2,948 ± 0,080<br>p< 0,01<br>p´< 0,02 | 3,784 ± 0,040<br>p < 0,05<br>p´< 0,001<br>p″< 0,001 | 3,352 ± 0,050<br>p< 0,02<br>p´> 0,05<br>p″< 0,001<br>p´″< 0,001 |
| ATP/AMP | 9,396 ± 0,800 | 6,034 ± 0,300<br>p<0,001 | 6,568 ± 0,200<br>p< 0,01<br>p´> 0,05 | 7,243 ± 0,200<br>p< 0,02<br>p´< 0,01<br>p″< 0,02 | 5,074 ± 0,070<br>p< 0,001<br>p´< 0,01<br>p″< 0,001<br>p´″< 0,001 |

Note: p - p-value for parameters in comparison to an intact group of animals; p´- compared to the group of physically exhausted animals without exposure to radiation, p″- compared to the group that started physical exercise 1 hour after exposure to irradiation, p´″- compared to the group that started physical exercise 24h after exposure to irradiation.

**Conclusions**

1. The total-body gamma irradiation at a dose of 154,8 mC/kg followed by intense physical exertion leads to a more significant suppression of the $Na^+$, $K^+$-ATPase activity and to a disturbance of the balance in the adenine nucleotide system in the heart muscle from rats in comparison with the animals that were subjected to exhaustive physical activity only.

2. Negative changes in the studied indicators are determined by the time that has passed after the total-body gamma irradiation and are phase-based with a tendency to worsen on the 3rd day of the experiment.

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**Author Contributions**

Conceptualization, (Vasilieva A.G.); methodology, (Dubna Ye.S.); formal analysis, (Dubna Ye.S.); data curation, (Dubna Ye.S.); writing—original draft preparation, (Dubna Ye.S.); writing—review and editing, (Dubna Ye.S.); supervision (Tereshchenko L.O.). All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement**

The experimental studies were carried out in the conditions of a chronic experiment in accordance with international standards of humane treatment of vertebrate animals and approved by the Ethics Committee of Odesa National Medical University (N7/21, 11 October 2021)

**Informed Consent Statement**

The data of experimental studies are given. Written informed consent from the patients was not necessary to publish this paper.

**Data Availability Statement**

The data presented in this study are available on request from the corresponding author.

**Conflicts of Interest**

The authors declare no conflict of interest.