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# EFFECTS OF $Mg^{2+}$, $Cd^{2+}$, $Cu^{2+}$ LOW CONCENTRATIONS AND IMMOBILIZATION STRESS ON THE ACTIVITY OF ADENOSINE DEAMINASE IN DIFFERENT ORGANS OF RABBITS

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Long-term restriction of motor activity is a factor that significantly complicates the processes of vital activity of the organism, accompanied by stress reaction and corresponding shifts in metabolism. The processes ensuring the change of immunity under stress depend on the activity of AMP-deaminase, 5'-nucleotidase, adenosine deaminase (ADA). Inosine, formed during deamination of adenosine activates the enzymes of lysis of necrotic masses, accelerates the maturation of connective tissue, increases the activity of antitumor and antioxidant enzymes. The result of the studies show, that with a 3 hour-immobilization of rabbits the activity of ADA in organs (liver, kidneys, brain) of animals decreases. Metal ions ($Cd^{2+}$, $Cu^{2+}$, $Mg^{2+}$) also affect the activity of ADA in rabbits organs, even at a concentration of 1 $\mu M$ ions, the activity of ADA is significantly reduced. The results obtained can be used to asses the immunological status of the organism.

***Keywords*:** adenosine deaminase, immobilization stress, metal ions, rabbit.

**Introduction.** The problem of a stress, adaptation and post-stressful violations nominated has reckoned with the most urgent problems of modern biology and medicine recently. It is conditioned by the fact that a scientific and technological revolution, complication of professional activity, an urbanization and acceleration of rate of life have caused a sharp increase of the psycho-emotional loading and the number of stressful situations against which members of modern industrial society come up [1].

During the investigations it has been shown that the processes providing changes of immunity under a stress depend on activity of 5'-nuceotidase, adenosine deaminase (ADA) and AMP-deaminase [2]. Adenine and adenosine-acting amino-hydrolase are important groups of enzymes responsible for the metabolic salvage of purine compounds. Several subclasses of these enzymes have been described and given current knowledge of the full genome sequences of many organisms, it is possible to identify genes encoding these enzymes and group them according to their primary structure [3]. These enzymes are constitutive components of purine metabolism and their impairment may cause serious medical disorders. In humans ADA deficiency is linked to severe combined immunodeficiency and as such the

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enzyme has been approved for the first gene therapy trial [4]. It is established that adenosine oppresses proliferation, differentiation and functions of immune cells. Extracellular adenosine is an important regulatory molecule, the concentration of which is low in norm, but it can be quickly increased with tissues damage inflammation or hypoxia. In damaged tissues and inflammatory processes of adenosine as a product of the decay of ATP along with it stimulates purinergic receptors [5].

Primary function of ADA is to remove toxic derivatives of adenosine and desoxyadenosine and protection of cells from apoptosis [6]. The importance of adenosine as a regulator of activity of adenilatecyclase and level cAMP as an action mediator of such nucleotides as cAMP, AMP, ATP has been shown. The adenosine increases the AMP level either through adenilatecyclase complex by cAMP, AMP, ATP or suppression of the activity of phosphodiesterase. There are observations that adenosine in low concentration suppresses and in higher one causes the AMP level increase particularly in the final concentration of 100 *mmol* adenosine activates an adenilatecyclase. There is an opinion that adenosine has different inhibitory effects becoming apparent in certain tissues. Adenosine is formed at dephosphoriation of AMP or at amination of inosine and its level in a cell depends on activity of enzumes of 5'-nucleotidase, AMP-deaminase and ADA. It has been revealed that AMP, ADP and ATP can inhibit activity of ADA by a feedback mechanism [7]. In their turn, AMP, ADP and ATP levels depend on the activity of mitochondrial enzymes, providing transformation of energy from ATP. Adenosine can be destroyed till inosine by adenosine deamination or as a result of adenosinekinase reaction, it can become AMP. The inosine formed at deamination of adenosine activates some enzymes to destroy necrotic masses, accelerates synthesis of purine nucleotides [2].

Moreover, it has been shown that the adenosine intracellular concentration is increased with oxidative stress [6]. Adenosine activates glutatione peroxidase, which is the central enzyme of antioxidant defense [8]. Immobilization stress is accompanied by an increase of intensity of free radical reactions that leads to metabolic shifts on the part of all types of metabolism, including purine [9]. Violation of the activity of enzymes involved in purine metabolism leads to the accumulation of defective enzymes in cells and biological fluids. Excess of these substrates may itself have a toxic effect on certain types of cells and tissues, an excess amount of substrate can also be metabolized in other biochemical pathways with the formation of toxic products [10]. A number of diseases due to violation of purine metabolism can be suspected on the basis of a change in the content of uric acid in the blood plasma and in urine, since uric acid is the end product of purine catabolism. The study of a number of microelements that determine the state of metabolism of purine is also of interest, although their pathogenetic significance remains unclear [11].

Besides, heavy metals ($Cu^{2+}$, $Mg^{2+}$, $Cd^{2+}$) are among the main pollutants entering the environment. They are characterized by high biological activity, the ability to accumulate in the organism without reducing toxicity. Increasing anthropogenic pollution of various ecosystems makes it relevant to study the mechanisms of the effects of heavy metals on living organisms. It is known that the toxicity of heavy metals is mainly determined by their inhibitory effect on enzyme activity [4]. Complexes of enzymes with heavy metal ions are stable, and inhibition assumes

irreversible character. Thus, heavy metal ions can form strong complexes with amino acids and other biomolecules [12]. The possibilities of enzymatic diagnostics of diseases associated with impaired purine metabolism make it advisable to study thoroughly the physicochemical properties of purine metabolism enzymes.

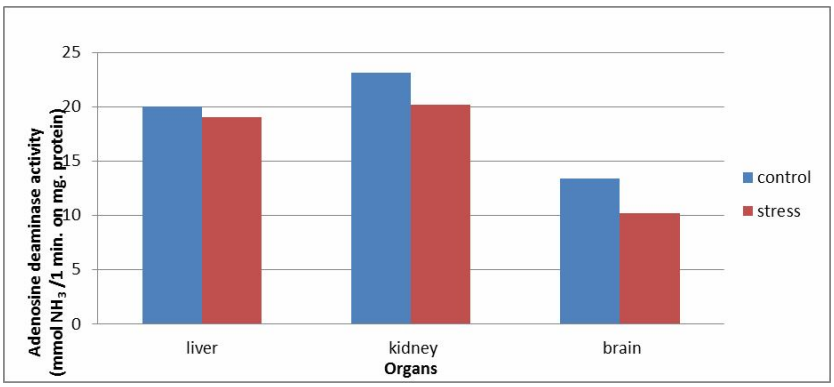
The aim of our research was to reveal changes of ADA activity in the norm and under the immobilized stress as well as influence of heavy metal ions on its activity in different organs (liver, kidney, brain) of rabbits. The study might be important to understand the role of ADA in cellular characterization of immune mechanism.

**Materials and Methods.** The objects of study were male rabbits (*Orictolagus cunculus domesticus*, 2000–2200 *g*), which were authorized by the "International Recommendation on Carrying out of Biomedical Researches with use of Animals" (the study plan has been approved by the National Center of Bioethics, Armenia). The rabbits were subjected to an immobilization stress and their different organs (liver, kidney, brain) were separated. 20% of homogenates have been prepared from the separated organs in 0.1 *M* K-phosphate buffer, pH 7.4 (Potter Elvenhjem homogenizer, duration 2–3 *min*). The homogenates were centrifuged during 15 *min* at 6000 *rpm*.

ADA enzyme activity was determined in supernatant of homogenate from the separated organs. Samples were incubated for 90 *min* at 37°*C*; the incubation mixture contained 1 *mL* homogenate, 0.5 *mL* adenosine (30 $\mu M$), 1.5 *mL* 0.1 *M* K-phosphate buffer (pH 7.4). The reaction was stopped by adding 1 *mL* of 20% trichloroacetic acid, after which the samples have been centrifuged for 10 *min*. Ammonia was measured in the supernatant by micro-diffusion technique of Selingson in the modification of Silakova [13].

Data processing was done using Excel 2013 Microsoft program.

**Results and Discussion.** Our investigations have shown that after 3 hour-immobilization the activity of ADA was decreased in the studied organs of rabbits. ADA levels were decreased in the investigated tissues under the influence of immobilization. From data presented it's seen, that 3 hour-immobilization stress in organ tissues of a rabbit the activity of ADA was decreased in kidneys on 12.6%, in brain was decreased on 24.3%, but it was less in liver on 5.3% (see Figure).




Dynamics of change of ADA activity in different organs of rabbits (ADA activity is expressed in *mmol* $NH_3$ formed at deamination of adenosine in 1 *min* per *mg* protein: $n = 7$; $p < 0.05$).

In the literature two immune deficiencies, connected with violation of the enzymes activity that control adenosine level in lymphocytes, are described [6]. The severe form of immunodeficiency caused by insufficiency of ADA is described, at the same time the quantity is decreased and function of both timus dependent lymphocytes and marrow lymphocytes are broken. There is an opinion that it is connected with increase of adenosine amount in lymphocytes, which can reduce the activity of adenylatecyclase or through blocking of phosphodiesterase [14].

It is of interest that the recent results demonstrated that serum ADA activity can be affected by different types of stresses [15]. The results of our experiments have shown that with a 3 hour-immobilization stress in the tissues of rabbit organs the activity of ADA was decreased, leading to the accumulation of adenosine, probably inhibiting the proliferation, differentiation and function of immune cells. A severe form of immunodeficiency due to ADA deficiency is described, while the quantity decreases and the functions of both T and B lymphocytes are disrupted. This indicates that the processes providing changes in the function of immuno-competent cells depend on the activity of 5'-nucleotidase, ADA and AMP-deaminase. The findings can be used in the diagnosis of various types of stresses, as well as in assessing the functional usefulness and condition of T- and B-links of immunity.

In addition, we have also studied the effects of ions of some bivalent metals on the activity of ADA in the studied organs (liver, kidney, brain) of rabbits (see Table). The data indicate that the metal ions inhibit the enzyme activity to various degrees. It is noteworthy that even at 1 $\mu M$ concentration of ions caused a drastic reduction with $Cd^{2+}$ inhibiting adenosine to a less degree. Most of the inhibition in ADA activity was observed in kidney tissues that can't be stated with $Cd^{2+}$ and $Cu^{2+}$ (see Table). In liver at the concentration of 5 $\mu M$ $Cd^{2+}$ and $Cu^{2+}$ the inhibition reached 58 and 71% respectively, and in brain $Cd^{2+}$ inhibited ADA activity for 45%, $Cu^{2+}$ – for 88% and more. Thus it is possible to note that heavy metal ions inhibited the activity of ADA. Moreover, inhibition by $Mg^{2+}$ was less than by $Cd^{2+}$ and $Cu^{2+}$. It should be noted that inhibition level was risen by the increase in concentration of specified ions.

Heavy metals ions in specific concentrations are in some cases essential for the activity. $MgCl_2$ and $CoSO_4$ had a remarkable activating effect on ADA from *Aspergillus terricola* [16]. $Fe^{3+}$ or $Sn^{2+}$ is promoting the ezymatic reaction of ADA in *Nocardiodies* sp. J-326TK and *Streptomyces* sp. For *Bacillus cereus* enzyme the activity is stable and it is stabilized by $NH^{4+}$ or $K^{+}$, while it is irreversibly lost in the absence of these or a few other monovalent cations [17]. Another known mammalian ADA from *Camelus dromedaries* is inhibited by $Mg^{2+}$, $Mn^{2+}$ and purine riboside [18].

Interestingly, extracellular adenosine is an important regulatory molecule, the concentration of which is low in norm; but its concentration can be quickly increased during excretion. Adenosine along with ATP stimulates purinergic recaptors. The release of ATP and adenosine, their signaling activity and subsequent depletion in the environment is the stage of an acute excretion process. An important stage in the regulation of adaptive immunity is the deamination by ADA. The main function of ADA is to remove toxic derivatives of adenosine and deoxyadenosine and protect cells from apoptosis [19]. Adenosine activates the glutation peroxidase 1, the central enzyme of the body's antioxidant defense. Increasing or decreasing the amount of adenosine can be an important adaptive

mechanism in oxidative stress, so the role of ADA as a regulator of adenosine levels is very important [20].

*Effects of heavy metal ions on ADA activity in the homogenates of organs in rabbits (the activity ADA is expressed in mmol $NH_3$ formed at deamination of adenosine in 1 min per mg protein, n=7, p<0.05)*

| Heavy metal ions, $\mu M$ | Liver | | Kidney | | Brain | |
|---|---|---|---|---|---|---|
| $Mg^{2+}$ | activity | inhibition, % | activity | inhibition,% | activity | inhibition, % |
| 0 | 20.05 | – | – | – | – | – |
| 0.5 | 18.86 ± 0.16 | 6.0 | 22.04 ± 0.02 | 5.0 | 13.11 ± 0.11 | 3.0 |
| 1.0 | 17.09 ± 0.08 | 14.8 | 21.12 ± 0.17 | 9.0 | 12.58 ± 0.15 | 6.0 |
| 2.5 | 16.92 ± 0.10 | 15.7 | 21.07 ± 0.12 | 9.0 | 12.70 ± 0.08 | 5.0 |
| 5.0 | 16.80 ± 0.16 | 16.3 | 21.00 ± 0.04 | 10.0 | 12.83 ± 0.06 | 4.0 |
| $Cd^{2+}$ | | | | | | |
| 0 | 20.05 | – | – | – | – | – |
| 0.5 | 15.85 ± 0.10 | 21.0 | 20.05 ± 0.11 | 13.3 | 11.27 ± 0.08 | 16.0 |
| 1.0 | 12.98 ± 0.12 | 35.0 | 16.15 ± 0.10 | 30.2 | 8.45 ± 0.06 | 37.0 |
| 2.5 | 10.48 ± 0.07 | 47.8 | 15.88 ± 0.14 | 31.4 | 8.60 ± 0.12 | 36.0 |
| 5.0 | 8.35 ± 0.09 | 58.4 | 15.50 ± 0.09 | 33.0 | 7.74 ± 0.07 | 43.0 |
| $Cu^{2+}$ | | | | | | |
| 0 | 20.05 | – | – | – | – | – |
| 0.5 | 14.44 ± 0.14 | 28.0 | 15.46 ± 0.10 | 33.0 | 6.50 ± 0.07 | 51.0 |
| 1.0 | 7.59 ± 0.09 | 37.8 | 8.65 ± 0.12 | 62.59 | 6.00 ± 0.02 | 55.3 |
| 2.5 | 6.30 ± 0.11 | 69.0 | 8.60 ± 0.09 | 63.0 | 3.00 ± 0.05 | 78.0 |
| 5.0 | 5.90 ± 0.12 | 71.0 | 7.30 ± 0.13 | 68.0 | 2.53 ± 0.01 | 88.6 |

**Conclusion.** Based on the results obtained, we can recommend the determination of ADA activity for characterization of a functional state of immunity for diagnostics.

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