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STUDY OF ADENOSINE DEAMINASE IN DIFFERENT ORGANS OF A RAT

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It has been demonstrated that the adenosine deaminase of homogeneous solutions of rat's organs is subject to inactivation under the influence of bivalent metals (Ca^{2+}, Cu^{2+}, Mg^{2+}), even though different levels of sensitivity to these metals are shown. Maximum inhibitory effect has been observed when adding Cu^{2+} ions to the environment.

***Keywords*:** inhibitors of adenosine deaminase, activity center, catalytic activity.

Introduction. Purine nucleoside (adenosine) is a biologically active compound with regulatory functions, is formed mainly intracellularly as a result of AMP dephosphorylation and hydrolysis of S-adenosyl-homocysteine (SAH). Adenosine is involved in the regulation of various biochemical and physiological functions, as well as the interaction of immune system cells, secretion of specific substances from different cells, including activated immune cells, as well as regulation of neuroendocrine, muscular, cardiovascular systems [1–3]. Nucleoside activates adenylate cyclase, which promotes the accumulation of cyclic AMP in the cells and decreasing the function of lymphocytes. Cyclic AMP is also involved in the regulation of blood flow in coronary vessels, slows platelet aggregation and increases synthesis of ATP in the myocardium [4].

High concentration of adenosine has inhibitory effect on the synthesis of S-adenosyl methionine, which leads to disruption of DNA methylation, thus to disruption lymphocyte proliferation processes. The normal level of Ado in the cells is quite low. The high level of Ado causes inhibition of ribonucleotide reductase, which leads to slowing down DNA and RNA biosynthesis and cell death. Maintaining of Ado normal level in the cells is provided by the presence of a powerful system of removing excess Ado using adenosine deaminase, which is the key enzyme of purine metabolism regulating the level of ammonia and adenosine in tissues.

Adenosine deaminase (also known as adenosine aminohydrolase, ADA) is an enzyme (EC 3.5.4.4), which is needed for the breakdown of adenosine from food and for the use of nucleic acids in tissues. Thus, participating in metabolism and, hence, in the regulation of such physiologically active substrate, adenosine deaminase plays an important role in maintaining the status of the living organism [5, 6].

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The enzyme is widely distributed in nature and is present in virtually all mammalian cells. ADA is also found in birds, bacteria and reptiles. The highest concentration of human adenosine deaminase is found in the tissues of the heart, brain, spleen, colon, kidney and lungs [7, 8]. Localization of ADA is predominantly cytoplasmic, but membrane-bound and extracellular forms of adenosine deaminase can also be found. Adenosine deaminase has a tertiary structure. ADA active site contains a zinc ion, which is located in the deepest spot of the active site and connected to five atoms of His15, His17, His214, Asp295 and to the substrate. In its active site the ADA also contains residue of histidine, cysteine and aspartic acid [9, 10]. The low activity of adenosine deaminase violates the proliferation and maturation of T and B lymphocytes and is accompanied by severe cellular and humoral immunodeficiency, which are probably the result of the accumulation of toxic concentrations of adenosine in the cells [11]. Children suffering from this pathology usually die at an early age from bacterial, viral or fungal infections [12]. It is obvious that the study of the ADA is one of the most promising approaches to understanding the physiology of certain pathological conditions. During the last thirty five years extensive research has also been conducted in the context of inhibitors of adenosine deaminase, which is conditioned by their ability to influence the Ado concentration in the tissues [13].

Materials and Methods. The objects of study were the organs (liver, kidney, brain) of rats. 20% homogenates of organs have been prepared have been prepared from the separated organs in 0.1 *M* K-phosphate buffer, pH 7.4 (Potter-Elvehjem, homogenizer, duration 2–3 *min*). The homogenate has been centrifuged for 10 *min* at the speed of 6000 *rpm*. Enzyme activity has been determined in supernatant of homogenate from the separated organs. Samples have been incubated for 60 *min* at 37°*C*. The incubation mixture contained 1 *mL* of homogenate, 0.5 *mL* adenosine (30 μM), 1.5 *mL* 0.1 *M* K-phosphate buffer (pH 7.4). The reaction has been stopped by adding 1 *mL* of 20% trichloroacetic acid, after which the samples have been centrifuged for 10 *min* at the speed of 5000 *rpm* and ammonia has been measured in the supernatant by microdiffusion technique of Selingson, modification of Silakova [14]. The method is based on the diffusion of ammonia supernatants by alkalization in a diffusion bottle using rotator followed by enzyme activity assay by a colorimetric method, based on measurement of the reaction of liberated ammonia with Nessler's reagent.

Results and Discussion. This article presents the results of a comparative study of the adenosine deaminase catalytic activity from tissues liver, kidney, brain of rats. Results indicate that adenosine deamination level in the kidney tissue is higher than in liver tissue and in brain (18.12; 15.31 and 10.32 μM respectively) (Tab. 1). The research shows that adenosine deaminase is a suitable target for the study of the catalytic function in the presence of a strongly-binding inhibitors, in particular Cu^{2+} ions [15, 16]. Based on this, we examined the effect of copper ions on the activity of ADA, as well as the inhibitory effect of divalent metal ions having lower covalent character (Mg^{2+}, Ca^{2+}). To this end, the homogenates of the examined tissues were incubated for 60 *min* at 37°*C* in the presence of different concentrations of these ions. The results presented herein refer to the enzyme activity at a concentration of 0.5 *mkm* to 2.5 *mkm* per trial, since the further increase in the concentration of used ions was not exacerbating the inhibitory effects on the activity of the enzyme.

Table 1

ADA activity in liver, kidney and brain homogenates of rat (NH_3 μM per 1 g of fresh tissue)

Object		
Liver	Kidney	Brain
15.31 ± 0.43	18.12 ± 0.71	10.32 ± 0.32

The data indicate that the metal ions inhibit the enzyme to various degrees. It is noteworthy that even at 1 μM concentration of ions cause a drastic reduction in the activity of enzyme in all tissues. However, a further increase in the concentration of metal ions in the incubation setting to 2.5 μM is not accompanied by significant changes in the activity of ADA. It should also be noted that the performance of inhibiting ADA by Cu^{2+} ions in all tissues exceeds that of other ions in the respective samples.

Table 2

The influence of different concentrations of Ca^{2+}, Mg^{2+} and Cu^{2+} on activity of ADA in the homogenates of rat's organs (NH_3 μM per 1g of fresh tissue)

		Object					
		Liver		Kidney		Brain	
		activity $\frac{\mu M}{mg \cdot h}$	inhib., %	activity $\frac{\mu M}{mg \cdot h}$	inhib., %	activity $\frac{\mu M}{mg \cdot h}$	inhib., %
Ca^{2+}	0.5	14.12 ± 1.49	8	17.04 ± 1.59	6	10.03 ± 0.27	2.8
	1.0	12.35 ± 0.58	19	16.12 ± 1.03	11	9.50 ± 0.81	7.9
	1.5	12.18 ± 0.39	20	16.07 ± 0.74	11	9.64 ± 0.47	6.5
	2.5	12.07 ± 1.04	21	16.10 ± 0.47	11	9.69 ± 0.33	6.1
Mg^{2+}	0.5	10.85 ± 1.49	29	15.05 ± 0.27	17	8.19 ± 0.82	21
	1.0	7.98 ± 0.36	48	11.15 ± 1.59	39	5.37 ± 1.59	48
	1.5	7.47 ± 1.02	51	10.88 ± 1.02	40	5.50 ± 0.27	47
	2.5	7.44 ± 0.79	51	10.62 ± 1.46	41	5.32 ± 0.34	48
Cu^{2+}	0.5	9.7 ± 1.46	37	10.5 ± 1.36	42	5.37 ± 1.25	48
	1.0	2.85 ± 0.48	81	3.69 ± 0.45	80	1.58 ± 0.83	85
	1.5	2.72 ± 0.88	82	3.76 ± 0.14	79	1.76 ± 1.02	83
	2.5	2.76 ± 0.42	82	2.92 ± 0.16	84	1.27 ± 0.51	88

According to obtained results (Tab. 2), at ion concentration of 1.0 μM ADA activity in rat's liver is inhibited by Ca^{2+} ions by 19%, Mg^{2+} ions by 48%, Cu^{2+} ions by 81%. ADA in rat's kidney is less sensitive to Mg^{2+} and Ca^{2+} ions (39% and 11% respectively), but Cu^{2+} ions inhibit ADA activity by 80%.

When Cu^{2+} ions were added into the incubation setting, ADA in the rat's brain was more actively inhibited (85% when $[Cu^{2+}]$ = 1.0 μM). Increasing the $[Cu^{2+}]$ to 2.5 μM practically leads to loss of ADA activity in the brain tissue (88%).

Published data also show significant suppression of ADA activity in various biological objects by Cu^{2+} ions. Particularly, Wilson et al., based on X-ray data, supposed the presence of several histidine residues in the active center mouse's

ADA, which allowed them to assume that the reason for the significant inhibition of the enzyme by Cu^{2+} ions can be a result of the interaction of the ions with the histidine residues in the ADA active center and the formation of a strong complex with it, which leads to the loss of enzyme activity [17].

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