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Analysis of Na+/K+ ATPase activity in selected brain structures of mice after intra-peritoneal injection of Streptozotocin

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ABSTRACT

The sodium-potassium pump (Na,K-ATPase) is a critical protein found in the membranes of animal cells. It functions in the active transport of potassium and sodium ions across the cell membrane against their gradients of concentrations **(Morth *et al., 2007).*** The aim of our work was to estimate the Na,K-ATPase activity in selected brain structures (right hemisphere, the left hemisphere, cerebellum and trunk of the brain) after streptozotocin. The level of ions was measured in the mixture of supernatant on the EasyLyte Na/K/Cl analyzer. We noticed influence of streptozotocin on the sodium-potassium pump activity in selected brain structures. Although no high significant changes after each time, we noticed decreased tendency in the activity. Significantly decreased of Na,K-ATPase activity occures in diabetic patients.

Key words: Na+/K+ ATPase, brain, streptozotocin, oxidative stress, diabetes

INTRODUCTION

Sodium-potassium adenosine triphosphatase (Na+/K+ ATPase) is also known as Na+/K+ pump, sodium pump or sodium-potassium pump.

The Na/K-ATPase is a critically and important membrane protein. This pump transports 2 K+ ions into and 3 Na+ions out of the cell against the electrochemical gradient by using the energy of the hydrolysis of 1 ATP molecule per transport cycle. Na+/K+ ATPase has many functions. It creates and maintains the transmembrane Na+ and K+gradients that contribute to the membrane excitability and potential, determining a significant fraction of the cellular metabolic rate via ATP hydrolysis and driving secondary active transport systems coupled to Na+ fluxes. 30–70 % of the cell's ATP is used for this transporter **(Hall *et al.,* 2006).** Furthermore, the Na/K-ATPase is the pharmacological receptor for cardiac glycosides.

In the last decade, many researches demonstrated different functions of the pump. In addition to the classical ion transporting, this membrane protein can also relay extracellular ouabain-binding signalling into the cell through regulation of protein tyrosine phosphorylation. Ouabains signals activate protein kinase (MAPK) signal cascades, activation of phospholipase C (PLC) and inositol triphosphate (IP3) receptor (IP3R) as well as mitochondrial reactive oxygen species (ROS) production in different intracellular compartments **(Howarth et al., 2012; Morth *et al.,*2007).** The sodium-potassium pump was discovered by Jens Christian Skou - Danish scientist in the 1950s. It was an important step to our understanding of how ions get into and out of cells. Moreover, it has a particular significance for excitable cells such as nervous cells, which depend on this pump for transmitting impulses and responding to stimuli (**Morth *et al.,*2007).**

The aim of our work was to estimate the Na,K-ATPase activity in selected brain structures after streptozotocin in: the: right hemisphere, the left hemisphere, cerebellum and trunk of the brain.

MATERIALS AND METHODS

The experiment was carried out on 24 male mice of Swiss strain. At the beginning, we determined the level of Na and K, then we calculated the ratio of Na/K which helped to show Na,K-ATPase activity.

The measurements were performed after 48, 72 hours, 8 days and 16 days after streptozotocin injection in single dose – 65 mg/kg b.w in: the: right hemisphere, the left hemisphere, cerebellum and trunk of the brain.

The level of ions was measured in the mixture of supernatant on the EasyLyte Na/K/Cl analyzer. Statistical analysis was performed using analysis of variances ANOVA. Homogoneity of variances was estimated using Wilcoxon signed rank test.

RESULTS

We noticed influence of streptozotocin on Na+/K+ ATPase activity in selected brain structures.

Moreover, we can notice many fluctuations in Na+/K+ ATPase activity. It was probably the result of the body's attempt to adapt to the inject dose of streptozotocin.

Taking into account Na/K ATPase activity there were no significant changes in the right hemisphere but we can notice decrease tendency of this activity (Fig.1). The mean of Na/K ATPase and standard deviation are shown in Tabele1.

Table 1.

Mean and Standard deviation of Na/K ATPase activity in the right hemisphere.

GROUPS AND TIME	MEAN [µMOL/L]	SD
48H		
Control RH	13,54	1,238
Streptozotocin RH	12,73	1,122
72H		
Control RH	12,40	2,756
Streptozotocin RH	9,401	1,935
8D		
Control RH	14,65	2,794
Streptozotocin RH	11,30	2,454
16D		
Control RH	16,48	1,465
Streptozotocin RH	16,79	2,678

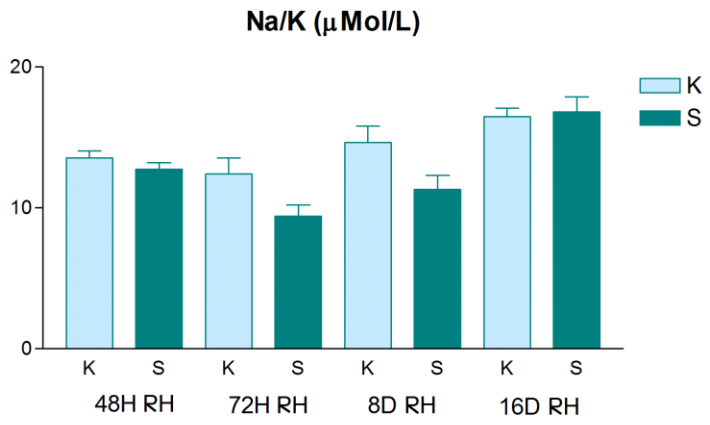

Fig.1.

Na/K-ATPase activity in the right hemisphere.

Taking into account Na/K ATPase activity there were no significant changes in the left hemisphere but we can notice decrease tendency of this activity too (Fig.2). The means of Na/K ATPase and standard deviation are shown in Tabele 2.

Table 1.

Mean and Standard deviation of Na/K ATPase activity in the left hemisphere

GROUPS AND TIME	MEAN [µMOL/L]	SD
48H		
Control LH	17,05	4,935
Streptozotocin LH	11,56	4,952
72H		
Control LH	14,98	4,787
Streptozotocin LH	15,83	5,256
8D		
Control LH	12,08	2,882
Streptozotocin LH	9,792	3,113
16D		
Control LH	18,90	3,780
Streptozotocin LH	15,45	2,836

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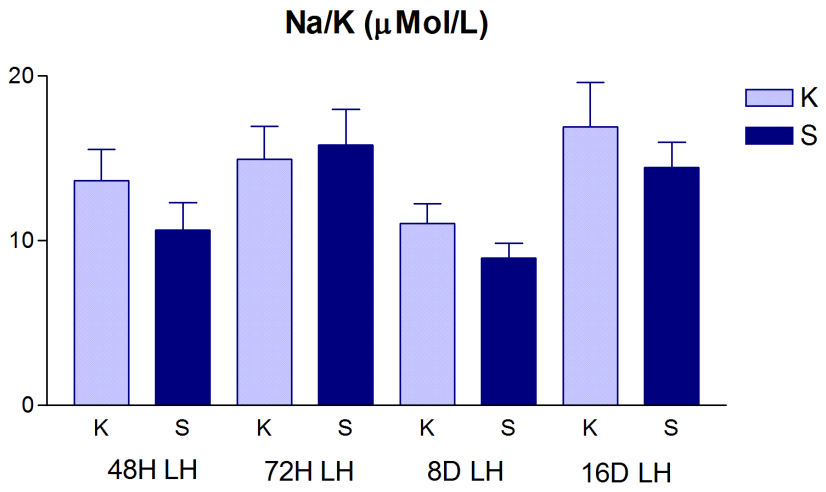

Fig.2. **Na/K-ATPase activity in the left hemisphere.**

Taking into account Na/K ATPase activity there were two significant changes in the cerebellum after 8 days (p=0,0313, r=0,4781) and after 16 days (p=0,0313, r=0,5429). Generally, we can notice decrease tendency of this activity (Fig.3). The means of Na/K ATPase and standard deviation are shown in Tabele 3.

Table 1.

Mean and Standard deviation of Na/K ATPase activity in the cerebellum.

GROUPS AND TIME	MEAN [µMOL/L]	SD
48H		
Control CB	25,20	6,103
Streptozotocin CB	27,51	6,680
72H		
Control CB	25,30	7,926
Streptozotocin CB	22,66	2,651
8D		
Control CB	25,57	5,151
Streptozotocin CB	20,84	5,840
16D		
Control CB	39,59	5,461
Streptozotocin CB	24,71	2,392

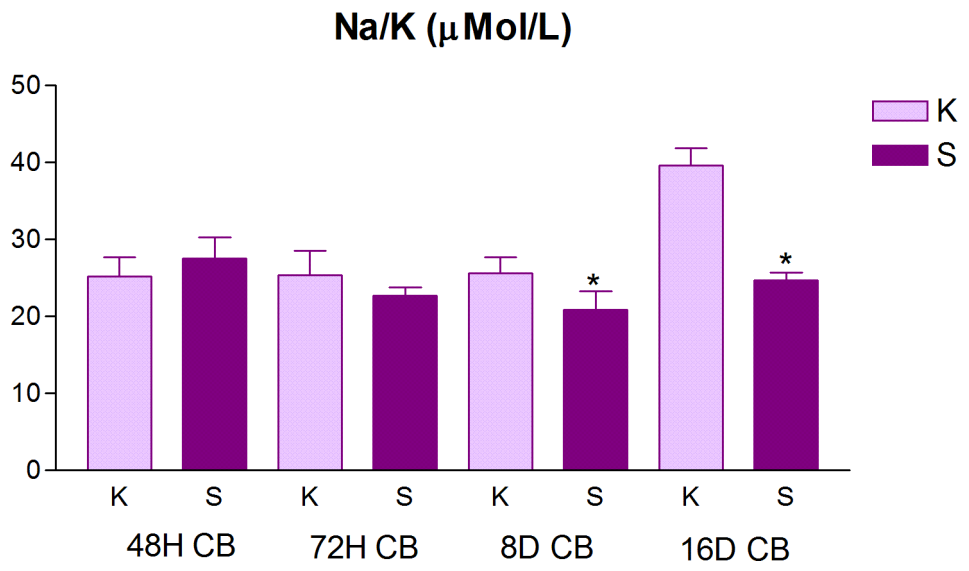

Fig.3.

Na/K-ATPase activity in the cerebellum.

Taking into account Na/K ATPase activity there were no significant changes in the trunk of the brain but we can notice decrease tendency of this activity (Fig.4). The means of Na/K ATPase and standard deviation are shown in Tabele 4.

Table 4.

Mean and Standard deviation of Na/K ATPase activity in the trunk of the brain.

GROUPS AND TIME	MEAN [µMOL/L]	SD
48H		
Control TB	35,90	10,87
Streptozotocin TB	25,43	5,528
72H		
Control TB	22,50	5,090
Streptozotocin TB	20,59	6,551
8D		
Control TB	25,57	5,151
Streptozotocin TB	19,31	5,674
16D		
Control TB	33,03	6,691
Streptozotocin TB	30,33	5,440

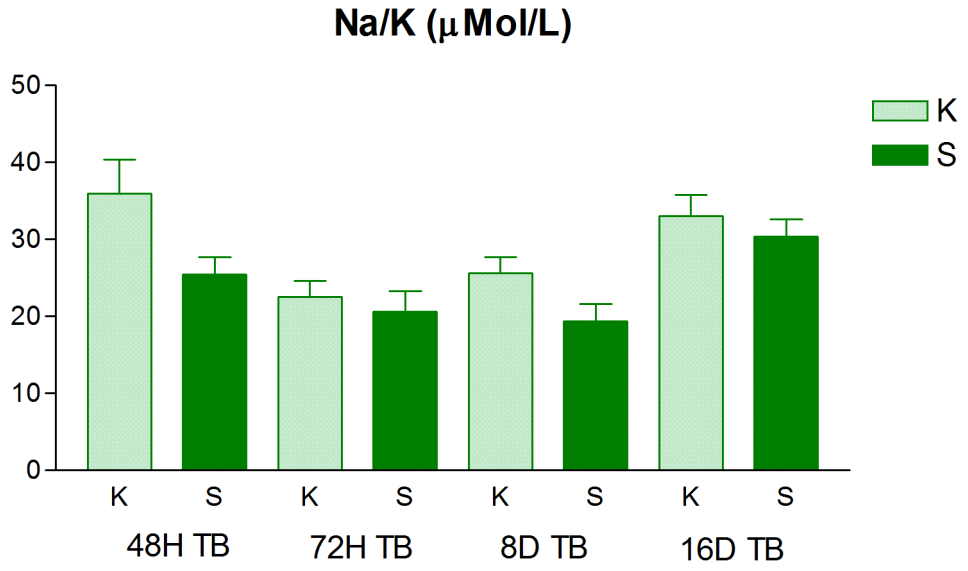

Fig.4.

Na/K-ATPase activity in the trunk of the brain.

DISCUSSION

We know the fundamental role of $K^+$and Na^+ transport in maintaining ionic gradients. This ions regulate cell metabolism and nervous system. Despite of this, our understanding of the regulation of Na/K ATPase remains incomplete.

Many researches on diabetes have based on the neuropathy of nervous system (**Górski *et al.,* 2000).** Moreover, that studies have shown that the NA/K ATPase in this system is reduced **(Flekac *et al.,* 2008).** Glucose utilization have been albo reduced. Glucose decrease is also associated with a reduced myo-inositol concentration in the nerve **(Drzewoski *et al.*, 2008).**

In contrast to many researches about peripherial nervous system, the changes in Na/K ATPase activity in selected brain structures are largely not known.

In order to defend against the damaging effects of free radicals your body has developed protective mechanisms in the form of antioxidant defense systems: non-enzymatic and enzymatic.

The enzyme system is responsible for antioxidant defense. This system is composed of superoxide dismutase, catalase and glutathione peroxidase **(Fraczek *et al.,* 2005).**
It was found that hyperglycemia promotes the formation of reactive oxygen species **(Mrowicka, 2011).**

Diabetes is associated with an increased level of free radicals and disturbances of the enzymatic antioxidant defense system. Furthermore, these abnormalities lead to a redox imbalance called oxidative stress **(Mrowicka, 2011).** The activity of sodium-potassium pump is impaired by oxidative stress. It is of great importance in the pathogenesis of diabetes. In normal conditions there is a balance between the emerging reactive oxygen species (ROS) and endogenous antioxidant systems. Oxidative stress is a condition characterized by excessive reactive oxygen species. As a result of elevated blood glucose increased occurs the impairment of remove free radicals. The blood vessels are and enhances the oxidative stress. It inhibits the sodium-potassium pump. In addition, the source of reactive oxygen species in diabetes is a mitochondrial respiratory chain **(Giugliano *et al.,* 1996).** It has been shown that increasing glycolysis and the Krebs cycle in response to hyperglycemia leads to a huge increase in the pool of protons, which then cause an increase in proton gradient and consequently there is a formation of free radicals. Sodium potassium pump activity is also reduced by decreasing of energy in cells **(Brownlee, 2001).**
Hyperglycemia also causes an increased conversion of glucose to sorbitol and fructose by the polyol pathway **(Dickinson *et al.,* 2002).** Decreasing ratio of reduced to oxidized form of nicotinamide adenine dinucleotide phosphate - NADPH / NADP +, and increases the ratio of nicotinamide adenine dinucleotide - NADH / NAD +, which leads to activation of growth cycle of transformations of arachidonic acid, which determines the additional production of free radicals **(Brownlee, 2001).**

The streptozotocin initiated first step of diabetes. It was the reason of starting production of free radicals and occurrenced oxidative stress. In our opinion, it was not enough time to induced full diabetes. We need to continue our experiment. It could be the reason of no significant changes in Na/K ATPase activity.

Although not many significant changes after each time, we noticed decreased tendency in Na/K ATPase activity. Significantly decreased of Na+/K+ ATPase occures in diabetic patients.

CONCLUSIONS

The present study has confirmed other reports decreasing activity of Na/K ATPase. Further studies involving streptozotocin and much time are necessary to clarify the results of the present study.

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