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DIFFERENCES IN Na^+, K^+, Cl^- IONS LEVEL AFTER INTRAPERITONEAL STREPTOZOTOCIN INJECTION IN CHOSEN BRAIN STRUCTURES

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ABSTRACT

Streptozotocin is an antibiotic which is used to induce experimental diabetes in experimental mice. Intraperitoneal administration of streptozotocin causes cytotoxic influence on different brain structures. This effect is associated with the generation of reactive oxygen species (ROS). Na^+, K^+, Cl^- play fundamental role of transport in maintaining the necessary ionic gradients for cell metabolism and excitability in the nervous system. The aim of our work was to estimate the level of the Na^+, K^+, Cl^- ions 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 the streptozotocin on the selected ions level. Although no high significant changes after each time, we noticed increased tendency in Na^+, K^+, Cl^- ions level. Significantly increased level of Na^+, K^+, Cl^- ions occures in diabetic patients.

Keywords: Na^+/ K^+/Cl^- ions, brain, streptozotocin, oxidative stress, diabetes

INTRODUCTION

Diabetes is a very serious threat for our health. Nowadays, the number of patients with this disease is increasing rapidly. Streptozotocin is the chemical which is particularly toxic to the insulin-producing beta cells of the pancreas in mammals **(Acharya, 2010).** Streptozotocin is used in medical research to produce an animal model for Type 1 diabetes in large dose as well as Type 2 diabetes with multiple low doses **(Szkudelski, 2001).** From chemically point of view, electrolytes are substances which become ions in solution and acquire the capacity to conduct electricity **(Hall, 2006).** Electrolytes are present in the human body, and the balance of the electrolytes in our bodies is essential for normal function of our cells and our organs **(Perzyna, 2011).** Electrolytes play fundamental role of transport in maintaining the necessary ionic gradients for cell metabolism and excitability in the nervous system. It's still little data about level of different ions in brain during diabetes.
The aim of our work was to estimate the level of the Na^+, K^+, Cl^- ions in selected brain structures after we were using the streptozotocin injection to test: the right hemisphere, the left hemisphere, cerebellum and trunk of the brain of the mice.

MATERIAL AND METHODS

The experiment was carried out on 24 Swiss mice, 8 week old, weight 26 g. The animals were segregated into 3 experimental and 3 control groups. The experiment has been done in Animal Facility of Department of Animal Physiology in Slovak University of Agriculture in Nitra. All the applied procedures were accepted by the Local Ethic Committee (27/III/2012). The measurements were performed after 48, 72 hours, 8 days and 16 days after intraperiotoneal streptozotocin injection in single dose – 65 mg/kg b.w in: the: right hemisphere, the left hemisphere, cerebellum and trunk of the brain. After each time of the experiment mice were anaesthetized and the structures of the brain trunk, cerebellum and both hemispheres were dissected.The level of ions was measured in the mixture of supernatant on the EasyLyte Na/K/Cl analyzer. Measured results are displayed and printed in 35 seconds on a 55 µL serum sample.

Statistical analysis

Obtained data were statistically analyzed using PC program GraphPad Prism 3.02 (GraphPad Software Incorporated, San Diego, California, USA). Descriptive statistical characteristics (mean, minimum, maximum, standard deviation and coefficient of variation) were evaluated. One-way analysis of variance (ANOVA) and the Wilcoxon's multiple comparison test were used for statistical evaluations. The level of significance was set at *** (P<0.001); ** (P<0.01) and * (P<0.05).

RESULTS AND DISCUSSION

This study was created to examine the effect of the streptozotocin on Na^+, K^+, Cl^- ions level of the in mice brain we confirmed the influence of the streptozotocin on this ions in selected brain structures. What is more, we noticed many fluctuations in of the Na^+, K^+, Cl^- ions level. It was probably the result of the body's attempt to adapt with to the inject the streptozotocin dose.

The effect of the streptozotocin on sodium level

The data of the below charts are indicate that the streptozotocin administration caused an increase of the sodium level in chosen brain structures. We can observe significant changes in the right hemisphere after 16 days, in the left hemisphere after 16 days, in cerebellum after 48 hours and in trunk of the brain after 16 days.

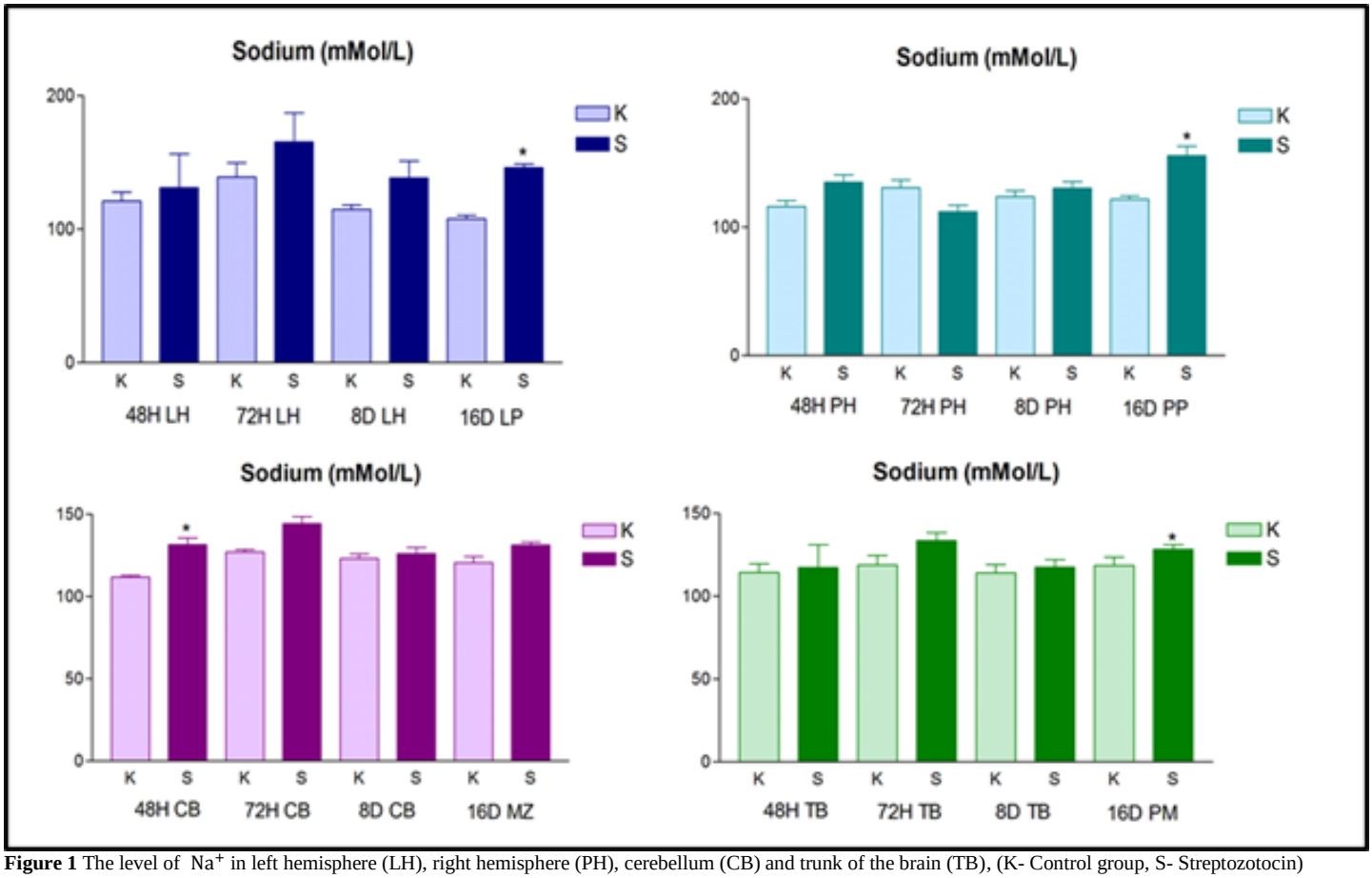

Figure 1 The level of Na^+ in left hemisphere (LH), right hemisphere (PH), cerebellum (CB) and trunk of the brain (TB), (K- Control group, S- Streptozotocin)

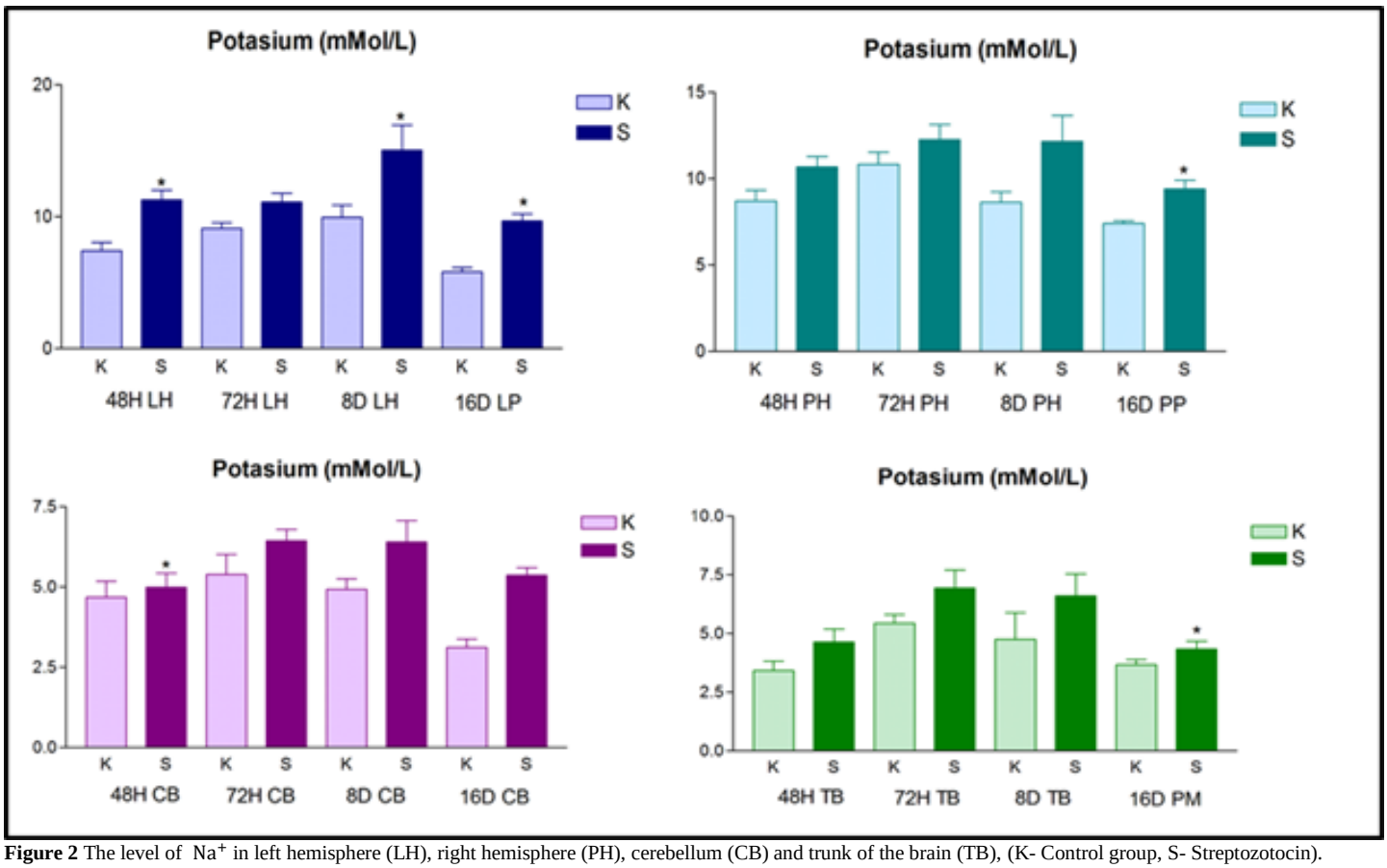

Figure 2 The level of Na^+ in left hemisphere (LH), right hemisphere (PH), cerebellum (CB) and trunk of the brain (TB), (K- Control group, S- Streptozotocin).

The effect of the streptozotocin on potassium level

As shown on the bar charts below, administration of the streptozotocin caused increase in potassium level in different brain structures. We confirmed significant changes in the right hemisphere after 16 days, in the left hemisphere after 48 hours, 8 and 16 days, in cerebellum after 8 and 16 days and in trunk of the brain after 16 days.

The effect of the streptozotocin on chloride level

The data of the below charts are indicate that streptozotocin administration caused an increase of the chloride level in chosen brain structures. We can notice significant changes in the right hemisphere after 48 hours, 8 and 16 days, in cerebellum after 48 and 72 hours and in trunk of the brain after 72 hours (p=0,0167) and 16 days (p=0,0292).

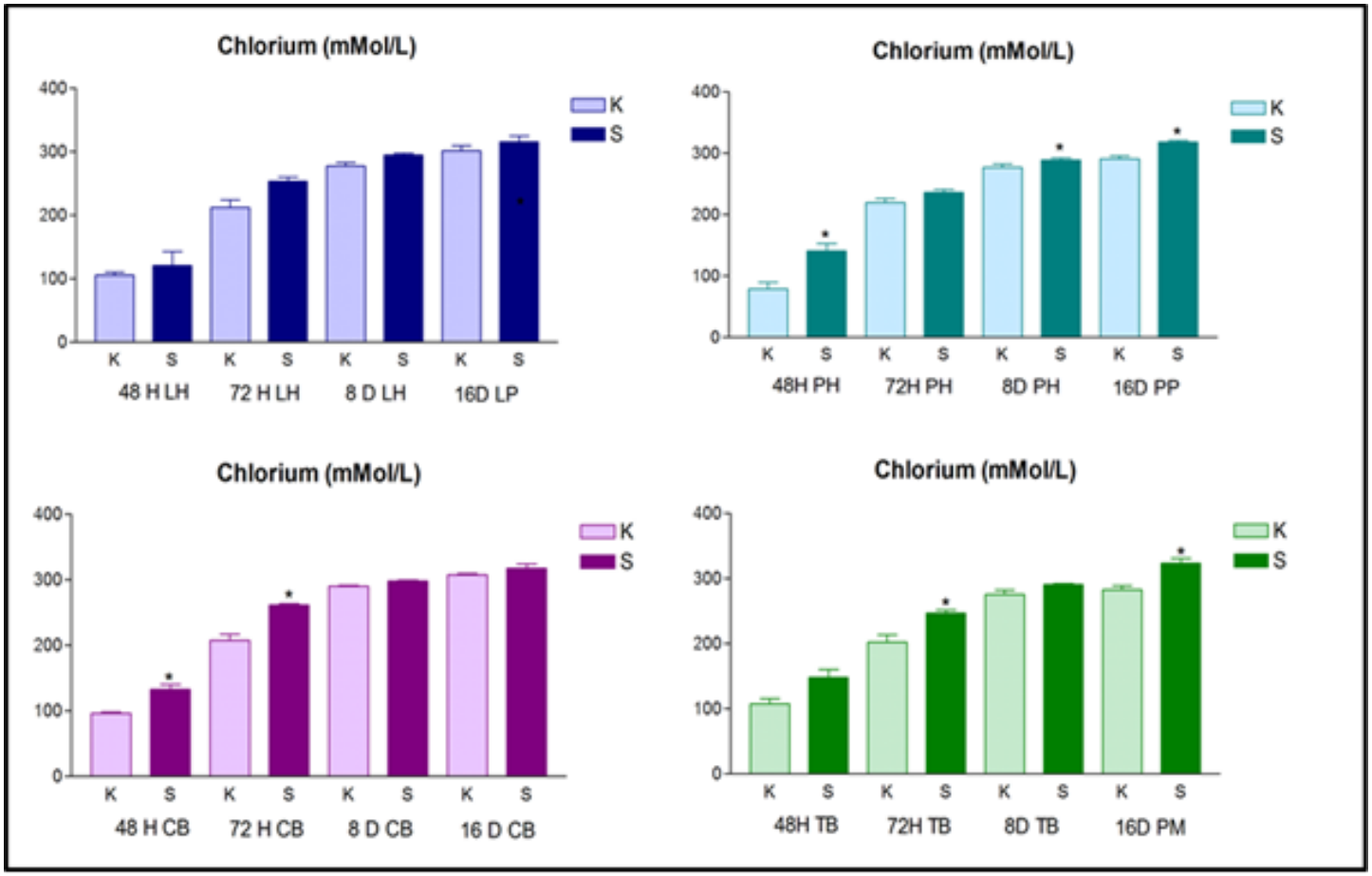

Figure 3 The level of Na^+ in left hemisphere (LH), right hemisphere (PH), cerebellum (CB) and trunk of the brain (TB), (K- Control group, S- Streptozotocin).

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

There are various researches on diabetes. It has been based on the neuropathy of the nervous system (**Górski *et al.*, 2000).** There are still little data about the influence of the streptozotocin on the different ions level. We concluded in our other studies **(Kopańska *et al.*, 2013)** that streptozotocin has influence on the Na/K activity. As we can noticed here, this toxic substance has also influence on the level of the Na^+/ K^+/Cl^- ions. From the previous researches which were carried on animal models of the streptozotocin we can say that the excessive production of the free radicals contribute to the pancreatic beta cells death **(Green et al., 2004).** In our organism we have special protective system non-enzymatic and enzymatic to defend against the damaging effects of the free radicals (**Fraczek *et al.*, 2005).** Suitable level of ions in our organism is very important for our protective system against oxidative stress.

As concluded by **Mrowicka (2011)** there are hyperglycemia promotes the formation of the reactive oxygen species and diabetes is also associated with an increased level of free radicals. Furthermore, these abnormalities lead to a redox imbalance **(Mrowicka, 2011).** Hyperglycemia is also the reason of increased conversion of the glucose to sorbitol and fructose by the polyol pathway **(Dickinson *et al.*, 2002).** In diabetes we can observe decreasing ratio of reduced to oxidized form of nicotinamide adenine dinucleotide phosphate - NADPH / $NADP^+$ as well as increases the ratio of nicotinamide adenine dinucleotide - NADH / NAD^+ It leads to activation of the growth cycle of transformations of arachidonic acid, which determines the additional production of free radicals **(Brownlee, 2001).**

Sodium regulates the total amount of the water in our body. The transmission of the Sodium into and out of individual cells is also very important in critical body functions **(McGuire, 2011).** Moreover, the proper level of potassium is crucial for normal cell function **(Campbell, 1987).** What's more, chloride also plays a role in helping the body maintain a normal balance of fluids **(Zumdahl, 2009).**

The streptozotocin initiated first step of diabetes. According to previous research **(Greń, 2012),** we can say that hyperglycemia in diabetic animals generates oxidative stress. It leads to a deficiency in activity of antioxidant defense system and causes an increase in *Reactive Oxygen Species* (ROS) levels **(Evans, 2003).**

From our results, we can conclude that 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 the levels of selected ions.

We can conclude that streptozotocin treatment increase ions level in selected brain structures. Moreover, we can notice many fluctuations in ions level. It was probably the result of the body's attempt to adapt to the inject dose of the streptozotocin. Although not many significant changes after each time, we saw increased tendency in level of Na^+/ K^+/Cl^- ions . After our experiment we confirmed that the streptozotocin has influence on selected ions level after each time and we noticed increased tendency in Na, K, Cl ions level. Moreover, changes in the concentration of studied ions probably reflect neurotoxic properties of the streptozotocin. We can also say that changes in the concentration of studied ions may also reflect changes activity on neurons system of mice.

CONCLUSION

The results of our studies confirmed that the observed changes in the level of Na^+, K^+, Cl^- in the different brain structures occur because of free radicals after administration of streptozotocin. Further, studies involving streptozotocin and much time are necessary to clarify the results of the present study.

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