

EFFECT OF MELATONIN ON FIBRINOLYSIS IN THE BRAIN OF RATS WITH DEXAMETHASONE DIABETES

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Summary

The purpose of our study was to determine the effect of melatonin on the characteristics of fibrinolysis in the brain of rats with dexamethasone diabetes.

Materials and Methods. The experimental study was carried out on thirty male eighteen-month-old non-linear white rats. The experimental rats were divided into three groups: 1) intact animals (control group); 2) rats with untreated dexamethasone diabetes; 3) rats, what were administered by melatonin in a dose of 10 mg per kg of body weight intragastrically daily through a metal probe during the 13 days of experiment in addition to dexamethasone injection.

Results. In the cerebral cortex of rats with diabetic neurodegeneration on the 14th day of the experiment, an increase in total fibrinolysis due to enzymatic fibrinolysis was found. Thus, TFA increased by 10%, and EFA by 15% compare to the indicators of animals of the control group. In the hippocampus, NFA indicators did not change, and EFA increased by 25% compared to the indicators of control group.

Daily oral administration of melatonin (10 mg/kg) to rats with diabetes-induced neurodegeneration did not significantly affect the EFA index in the cerebral cortex, but contributed to a 15% decrease in this indicator in the hippocampus, compared to the indicators of diabetic rats that did not receive any correction agents. The indicators of TFA and NFA both in the cerebral cortex and in the hippocampus were not significantly different from the indicators of rats with diabetes.

Conclusions. A decrease in the intensity of the fibrinolysis process was noted in the cerebral cortex and hippocampus of rats that, in addition to dexamethasone injections, were orally administered melatonin daily for 13 days.

Key words: fibrinolysis, brain, steroid diabetes, experimental study.

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1. Introduction

Today, there is a lot of evidence that the cascade of pathological changes that lead to the development of amyloid plaques and neurofibrillary tangles in neurodegeneration can be caused by the progression of type 2 diabetes. In particular, it is known that diabetes has a negative effect on cerebral metabolism, contributes to cerebral atrophy and accelerates the aging

of the brain. One of the main complications of diabetes mellitus (DM) is a decline in cognitive functions. At the same time, diabetes is one of the risk factors for the development of Alzheimer's disease, one of the most common neurodegenerative diseases (*M.M. Teixeira et al., 2020*).

The effect of chronic hyperglycemia can have a more pronounced effect than acute. It can be mediated by the formation of glycosylation end products, activation of alternative polyol and hexose metabolic pathways, activation of protein kinase C and inflammatory processes in the brain (*O Albai, M Frandes, 2019*). Overall, hyperglycemia certainly plays a role in the development of cognitive dysfunction. Cerebrovascular or neurodegenerative pathologies, which are accelerated by metabolic disorders characteristic of diabetes, play a more important role in the development of cognitive decline.

Unfortunately, cognitive impairment in patients with diabetes progresses very quickly and is often accompanied by the development of vascular dementia. It not only reflects the severity of diabetes, but may also be directly related to insulin therapy (*Arnold, S. E., Arvanitakis, Z., 2018*). The risk of neurodegeneration and cognitive deficits increases in insulin-resistant patients who do not have severe hyperglycemia.

A more important role in the development of cognitive decline was played by microvascular damage to the deep parts of the brain, which are supplied with blood by penetrating arteries. The causes of damage to small cerebral vessels in diabetes are not fully known. It is assumed that the formation of end products of glycosylation, as the activation of alternative pathways of carbohydrate metabolism, contributes to the development of oxidative stress, which leads to damage to the vascular endothelium and the development of brain ischemia (*Mykhailychenko T. E., Volos L. I., 2020*).

Already at the initial stages of diabetes, hemorheological disorders are noted, which are expressed in an increase in blood viscosity and the aggregation ability of erythrocytes and platelets, as well as in a decrease in fibrinolytic activity and an increase in the Willebrand factor in blood serum. Morphological progression of micro- and macroangiopathies is characterized by increased adhesion of leukocytes and platelets, deposition of fibrin on the endothelium, increased proliferation of endotheliocytes, thickening of the basement membrane, and increased permeability of the capillary wall (*Usmanova D. D., Khazhibakiev Kh. Kh., 2016*). Which generally disrupts the blood supply to the brain and worsens the state of neurodegenerative processes.

Medicines with antioxidant properties include melatonin, which, in addition to its regulatory action in the light-dark cycle, is a hormone with neuroprotective, anti-inflammatory, and antioxidant properties (*Zherdyova N.M., 2017*).

The goal of this study was to find out the influence of melatonin on the fibrinolytic activity, total fibrinolytic activity, enzymatic and non-enzymatic fibrinolytic activity in the brain of rats with dexamethasone diabetes.

2. Materials and methods

The experimental study was carried out on thirty male eighteen-month-old non-linear white rats. The experimental animals were divided into three groups: 1) control (intact animals); 2) rats with diabetes; 3) rats to which, in addition to dexamethasone, melatonin (Sigma, USA) in a dose of 10 mg/ kg was administered intragastrically daily during the experiment through a metal probe.

Diabetes was induced in rats according to the previously described method (*Kolesnyk Y. M., Ivanenko T. V., Abramov A. V., Kuzio N. V., 2016*), by daily subcutaneous injection

of dexamethasone at a dose of 0.125 mg/kg of the animal's body weight for 13 days. To induce the specified model of diabetes and the development of insulin resistance dexamethasone solution for injections – 4 mg/ml (KRKA, Slovenia) was used. Blood was taken from the tail vein to assess glycemia level using OneTouchUltra (LifeScan, USA). Euthanasia of animals was carried out in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (*Strasbourg, 1986*).

We chose the cerebral cortex and the hippocampus, which are responsible for the realization of the cognitive function, for conducting experimental studies.

The cytoplasmic fraction was isolated by the method of differential centrifugation of the homogenate of the cerebral cortex and hippocampus in a refrigerated centrifuge at 1000 g for 10 min, then 1400 g for 10 min at a temperature of 4 °C.

Study of tissue fibrinolysis and unlimited proteolysis. The study of biochemical markers of neuronal pathology involves determining the state of proteolysis/fibrinolysis. Fibrinolytic activity (FA) was determined based on the reaction with azofibrin (Simko Ltd., Lviv), that is, fibrin associated with an orange azo dye, which gives a bright red color in an alkaline medium (*Jayaraj RL, Azimullah S, Beiram R, 2020*). Total fibrinolytic activity (TFA), enzymatic (EFA) and non-enzymatic fibrinolytic activity (NFA) were also determined (*Liu J, Chang L, Song Y, Li H and Wu Y, 2019*).

The reliability of the difference between the obtained indicators was assessed using the parametric Student's t-test (for normal distribution) and the non-parametric Mann-Whitney U-test (for non-normal distribution). Differences were considered probable at $p \leq 0.05$.

3. Results

In the cerebral cortex of rats with diabetic neurodegeneration on the 14th day of the experiment, an increase in total fibrinolysis due to enzymatic fibrinolysis was found (Table 1). Thus, TFA increased by 10%, and EFA by 15% compare to the indicators of animals of the control group. In the hippocampus, NFA indicators did not change, and EFA increased by 25% compared to the indicators of control group.

Daily oral administration of melatonin (10 mg/kg) to rats with diabetes-induced neurodegeneration did not significantly affect the EFA index in the cerebral cortex, but contributed to a 15% decrease in this indicator in the hippocampus, compared to the indicators of diabetic rats that did not receive any correction agents. The indicators of TFA and NFA both in the cerebral cortex and in the hippocampus were not significantly different from the indicators of rats with diabetes.

Administration of dexamethasone to old rats, according to literary sources (*Jia-Xu Li, Carolyn L. Cummins, 2022*), leads to the development of insulin resistance and pathological changes in the tissues of rats, which are similar to those in type II diabetes.

An increase in the intensity of fibrinolysis in rats with dexamethasone diabetes can probably be considered as a compensatory reaction of the body to hypercoagulation associated with the probable accumulation of a pathological peptide in the brain, the so-called beta-amyloid, which leads to the development of neurodegeneration (*Chatterjee, S., & Mudher, A., 2018*).

A decrease in the intensity of the fibrinolysis process was noted in the cerebral cortex and hippocampus of rats that, in addition to dexamethasone injections, were orally administered melatonin daily for 13 days on the 14th day of the experiment.

Table 1

The influence of melatonin on fibrinolysis indicators in the cerebral cortex and hippocampus of rats with dexamethasone diabetes (n=10, $\bar{x}\pm S\bar{x}$)

Indicators	Brain structures	Control group	Dexamethasone diabetes	Dexamethasone diabetes + melatonin
Total fibrinolytic activity (μg of azofibrin/g of tissue per hour)	Cortex	82,11 \pm 1,31	93,34 \pm 2,68*	89,44 \pm 1,37*
	Hippocampus	50,43 \pm 1,65	54,73 \pm 2,98	51,35 \pm 1,92
Non-enzymatic fibrinolytic activity (mcg of azofibrin/g of tissue per hour)	Cortex	53,41 \pm 1,26	59,32 \pm 1,24	58,93 \pm 1,17
	Hippocampus	29,62 \pm 1,87	30,17 \pm 2,34	39,93 \pm 1,23
Enzymatic fibrinolytic activity (mcg of azofibrin/g of tissue per hour)	Cortex	26,82 \pm 0,56	31,22 \pm 0,41*	28,37 \pm 1,32
	Hippocampus	21,88 \pm 0,74	27,32 \pm 1,12*	21,19 \pm 1,78**

Notes: * – the reliability of the differences compared to the control group of rats; ** – significance of differences compared to the group of rats with dexamethasone diabetes.

The positive effect of melatonin on indicators of the fibrinolytic system is probably mediated by its antioxidant effect (*Russel J. Reiter, Ramaswamy Sharma, Sergio Rosales-Corral, (2021)*).

According to modern researchers, the pathogenesis of neurodegenerative diseases is closely related to oxidative stress, which initiates an inflammatory response and the accumulation of β -amyloid (*Kmet O., 2021*).

Reduction of fibrinolysis processes under the influence of melatonin improves the rheological properties of blood, reactivity of blood vessels, and compromise of the integrity of the endothelial layer. As a result, fibrinogen deposition processes are slowed down, inflammation is reduced and vascular permeability is normalized. The described mechanisms help to slow down the processes of vascular damage and, as a result, prevent neuronal dysfunction.

4. Conclusions

We have found a decrease in the intensity of the fibrinolysis process was noted in the cerebral cortex and hippocampus of rats that, in addition to dexamethasone injections, were orally administered melatonin daily for 13 days.

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