HEALTH, ENVIRONMENT, DEVELOPMENT

FIBRINOLYTIC ACTIVITY IN HEART TISSUE IN HYPOTHYROID AND HYPERTHYROID RATS UNDER THE INFLUENCE OF EXOGENOUS MELATONIN AND BLINDING CONDITIONS (ENUCLEATION)

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Summary

The aim of our scientific work was to study the effect of exogenous melatonin on the indicators of fibrinolytic processes in the heart tissues and to analyze the changes in fibrinolytic activity occurring in the heart tissues of enucleated hyper- and hypothyroid white rats.

The experiments were carried out on white nonlinear male rats weighing 0.12-0.14 kg. 4 experimental groups of animals were formed. The control group consisted of 11 normothyroid rats, which were injected with a melatonin solution in appropriate volumes. The animals were euthanized under light ether anesthesia. Samples of the examined heart tissue were ground in a glass homogenizer with borate buffer (pH 9.0). The homogenate was used in biochemical analysis. Determination of the total, enzymatic and non-enzymatic fibrinolytic activity (TFA, EFA, NFA, respectively) in the heart tissues was carried out by the method of azofibrin lysis (LLC "Simko", Ukraine).

Statistical processing of the results was carried out by the method of variation statistics using the Student's test.

Experimental studies on nonlinear male white rats have shown that the introduction of exogenous melatonin, as well as modeling the conditions of endogenous melatonin overproduction by blinding (enucleation) cause an increase in the intensity of enzymatic and non-enzymatic fibrinolysis in heart tissue in normotyroid animals. At other hand, enucleation causes an increase in the intensity of fibrinolysis in the tissues of the heart in hypothyroid rats, but not in hyperthyroid.

Keywords: exogenous melatonin, fibrinolytic activity, hypothyroidism, hyperthyroidism, enucleation, heart tissues

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1. Introduction

It is known that the pineal gland is a neuroendocrine formation that promotes the transformation of environmental signals into humoral stimuli and which is able to regulate the functioning of the hypothalamic-pituitary-thyroid complex. The pineal gland is a producer of compounds of the methoxindole family, of which N-acetyl-5-methoxytryptamine (melatonin) and 5-methoxytryptamine have hormonal properties and functions of endocrine rhythm synchronizers, including the thyroid gland *(Anysymov, 2006; Tan, 2010)*.

Endogenous regulation of melatonin production is carried out by suprachiasmatic nuclei (SCN), and external correction – by the photoperiod. It is known that melatonin production varies with a number of pathological conditions, in particular there are reports of increased melatonin levels in patients with cirrhosis of the liver, with chronic kidney and cardiovascular diseases *(Zaslavskaya, 2005)*.

Some researches shown that thyroid C-cells synthesize melatonin under thyroid-stimulating hormone control. Also, it is shown the involvement of melatonin in thyroid function by directly-regulating thyroglobulin gene expression in follicular cells *(Garcia-Marin, 2015)*. Administration of melatonin in dogs decreases concentration of T3 and T4 hormones and balance other metabolic hormones following castration. Also, is known, that exogenous MLT modified the progression of autoimmune thyroiditis through T cell-driven immunity, and excess MLT worsened the clinical and pathological features *(Lin, 2019)*.

In mammals, the light cycle is perceived by the retina, from there the nerve signal via the retino-hypothalamic pathway enters the SCN, and later in the upper cervical ganglion. From the latter, information about the light enters the pineal gland: it is mediated by norepinephrine (HA), which is released by nerve endings directly into the parenchyma of pineal cells, which leads to the start of melatonin synthesis *(Zhao Dake, 2019)*. Violation of the structure of chronorhythms (desynchronosis) is an indicator of the pathological state of the organism *(Komarov, 2000)*.

Melatonin has significant effect on myocardial structure and function. It induces cardiomyocyte proliferation and heart regeneration after myocardial infarction by regulating the miR-143-3p/Yap/Ctnnd1 signaling pathway, providing a new therapeutic strategy for cardiac regeneration *(Ma, 2021)*.

Particularly threatening is the imbalance of the chronorhythms of interdependent or cascading enzymatic reactions. Issues of fibrinolysis attract the attention of a wide range of clinical and theoretical medical professionals *(Litvinov, 2013)*. Depression of fibrinolytic activity is one of the pathogenetic factors in the development of thrombosis *(Violi, 2013)*. Statistics of myocardial infarction clearly show the daily dependence of this pathology, which may develope due to circadian fluctuations in fibrinolytic potential *(Ostrowska, 2004)*.

Given the above, we considered it appropriate to research the effect of exogenous melatonin, and the combined effect of melatonin hyperproduction on fibrinolytic activity in heart tissue in hypo- and hyperthyroid rats.

2. The aim of the study

The aim of this study is to research the effect of exogenous melatonin on the indexes of fibrinolytic processes in the heart tissues and to analyze changes in fibrinolytic activity occurring in the heart tissues of enucleated hyper- and hypothyroid white rats.

3. Material and methods

The experiments were performed on male nonlinear white rats weighing 0.12-0.14 kg. Melatonin was administered once intraperitoneally at a dose of 6 mg / kg body weight *(Anokhina, 2004)*. Enucleation (blinding) of rats was performed under nembutal anesthesia (at a dose of 40 mg / kg body weight), 0,1% dicaine solution was injected into the conjunctival sac, after which the eyeball was removed (group 1) *(Kuchuk, 2001)*. Hypothyroidism was caused by the introduction of mercazolyl at a dose of 10 mg / kg body weight for 10 days (group 2). Group 3 – enucleated hypothyroid rats. Simulations of hyperthyroidism were performed by daily intragastric administration of L-thyroxine at a dose of 200 μg / kg body weight for 14 days (group 4) *(Perepeliuk, 1992)*. Group 5 – enucleated hyperthyroid rats. The control group consisted of 11 normothyroid rats, which were injected with a solvent of melatonin in appropriate volumes. Euthanasia of animals was performed under light ether anesthesia. Samples of the test heart tissues were ground in a glass homogenizer with borate buffer (pH 9.0). The homogenate was used in biochemical analysis. Determination of the total, enzymatic and non-enzymatic fibrinolitic activity (TFA, EFA, NFA, respectivelly) in the heart tissues was performed according azofibrin lysis ("Simko Ltd", Ukraine) *(Kukharchuk, 1996)*.

Statistical analysis of the results was performed by the method of variational statistics using Student's test.

Experiments have been carried out in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes *(Strasbourg, 1986)*.

4. Results

It was found that exogenous melatonin causes an increase in total fibrinolytic activity in cardiac tissue, which was observed due to increased enzymatic fibrinolysis (by 37%) and non-enzymatic lysis of fibrin (by 31%) (Table).

Analysis of changes in tissue fibrinolysis in the hearts of blinded rats showed an increase in total fibrinolytic activity by 3,4 times, with symultanous increase in non-enzymatic fibrinolysis by 3,4 times, and EFA – by 3,3 times.

When administered to blinded animals mercazolyl, TFA increased relative to control by 3.8 times, due to an increase in NFA in 3.7 times, EFA – in 3.9 times. Compared to the group 1, the total fibrinolytic activity increased by 12%, due to an increase in enzymatic fibrinolysis by 17%. Relative to the group 2, the TFA indicator in the group 3 was 1,5 times higher due to an increase in non-enzymatic fibrinolysis by 1,5 times, and the enzymatic fibrinolysis – by1.6 times.

In case of L-thyroxine was administered to enucleated animals (group 5), the TFA increased 1,8-fold relative to the control, the NFA increased 1,7-fold, and the EFA increased 1,8-fold. Compared with the indicators of the group 1, the total fibrinolytic activity decreased by 1,9 times, due to the inhibition of enzymatic fibrinolysis by 1,8 times, and non-enzymatic one – by 1,9 times. Compared with the group 4, the total fibrinolysis in the heart tissues of rats in group 5 decreased by 2.1 times, due to a decrease in both enzymatic and non-enzymatic lysis of fibrin.

The obtained results indicate an increase in fibrinolysis in the myocardial tissue in rats of the third study group, which is carried out by increasing both enzymatic and non-enzymatic fibrinolysis. In our opinion, the obtained indicators are due to the combined effect of this indolamine, which is produced constantly in blinded rats *(Anokhina, 2002),* and the suppression of thyroid function. At the same time, there was observed an inhibition of fibrinolytic activity in hyperthyroid enucleated rats, which may demonstrates pineal-thyroid dependence. As it is known that melatonin is metabolized in the liver, excreted by the kidneys, and the intensity of these processes depends entirely on the state of the cardiovascular system, which can determine the characteristics of the latter on the indicators of tissue fibrinolysis. Also it is known that the distribution of exogenous melatonin in the body has features: the highest concentrations of this hormone are registered in the organs of the gastrointestinal tract, heart and blood plasma. In addition, each target organ has its own rhythm of melatonin sensitivity *(Arushanyan, 2016).*

Table

Characteristics of fibrinolytic activity in heart tissue under the influence of exogenous melatonin in normothyroid rats, and blinding in hypothyroid and hyperthyroid rats (x ± Sx)

№ of group	Applied influence / characteristic of group, n	TFA, мкg azofibrin / 1 g of tissue per 1 hour	NFA, мкg azofibrin / 1 g of tissue per 1 hour	EFA, мкg azofibrin / 1 g of tissue per 1 hour
Control 1	Normothyroid sighted rats, n=10	8,56±0,47	4,62±0,28	3,95±0,21
Control 2	administration of melatonin in normothyroid sighted rats, n=7	11.47+0,62 $p<0,005$	6,05±0,33 $p<0,01$	5,42+0,40 $p<0,005$
1	Enucleation, n=7	29,43±0,89 $p_1<0,001$	16,11±0,31 $p_1<0,001$	13,31±0,61 $p_1<0,001$
2	administration of merca-zolyl, n=7	21,06±1,15 $p_1<0,001$	11,51±0,59 $p_1<0,001$	9,56±0,57 $p_1<0,001$
3	administration of mer-cazolyl to enucleated animals, n=7	33,06±0,21 $p_1<0,001$ $p_2<0,001$	17,48±0,11 $p_1<0,001$ $p_3<0,001$	15,58±0,09 $p_1<0,001$ $p_2<0,01$ $p_3<0,001$
4	administration of L-thyrox-ine, n=7	33,75±1,81 $p_1<0,001$	17,90±0,91 $p_1<0,001$	15,85±0,90 $p_1<0,001$
5	administration of L-thyrox-ine to enucleated animals, n=7	15,48±0,69 $p_1<0,001$ $p_2<0,001$ $p_3<0,001$	8,22±0,45 $p_1<0,001$ $p_2<0,001$ $p_3<0,001$	7,26 ±0,24 $p_1<0,001$ $p_2<0,001$ $p_3<0,001$

Note in table: n – the number of observations; p – criterion of probability of difference of group control 1 in comparison with control 2; p1 – criterion of probability of difference in comparison with group control 1; p2 – criterion of probability of difference in comparison with group 1; p3 – criterion of probability of difference in comparison with group 4.

According to the results of this study, in the heart tissues the intensity of enzymatic fibrinolysis under the influence of exogenous melatonin increases. Melatonin is metabolized in the liver, excreted by the kidneys, and the intensity of these processes depends entirely on the state of the cardiovascular system, which may determine the characteristics of the latter on the indicators of tissue fibrinolysis.

5. Conclusion

Under the action of exogenous melatonin and the modelling of the hypothyroid state in the heart, there is a total activation of fibrinolysis, which is resultes by increasing of activity of both enzymatic and non-enzymatic fibrinolysis. Under the conditions of administration of L-thyroxine to enucleated rats, a decrease in fibrinolytic activity in heart tissue was found in comparison with the corresponding indicators of hyperthyroid sighted (non-enucleated) rats.

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