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THE EFFECT OF TRACE ELEMENT STATUS OF RATS WITH SIMULATED HYPOTHYROIDISM ON THE STATE OF CONGITIVE FUNCTIONS

Abstract. *Hypothyroidism is the second endocrine disease after diabetes mellitus associated with disorders of hormonal homeostasis, imbalance in the content of biological elements, decrease of motor activity, weight gain, dysfunctions of the central nervous system, cognitive functions in particular, and as a result decrease the quality of life. 42 albino male Wistar rats were used in the study. Their body mass (BM), level of thyroid hormones, concentration of copper, zinc and magnesium in the blood serum were evaluated. The state of cognitive functions was determined by means of the tests "Open field", "Social cognition" and detection of "New object". BM of rats with hypothyroidism was found to be more than 25% (p<0,05) as much. T3 and T4 levels in animals with hypothyroidism was found to decrease in 3,9 and 3,3 times (p<0,05), and the content of TSH in the blood serum increased twice as much. The concentration of copper in the blood of animals with hypothyroidism1,54 times increased (p<0,05), magnesium – 23,36% (p<0,05), and zinc – 6,8% (p>0,05). Under conditions of hypothyroidism development with a longer period of the experiment cognitive-learning functions of animals progressively decreased, as well as memory disorders concerning new and familiar objects or subjects which is indicative of the formation of stable cognitive deficiency.*

Key words: *hypothyroidism, cognitive functions, trace elements, experiment, rats.*

Introduction. Hypothyroidism is one of the most spread endocrine diseases after diabetes mellitus. It is a clinical syndrome associated with continuous decrease of the thyroid hormones action on the target systems. Against the ground of hypothyroidism practically all the organs and systems are afflicted making clinical manifestation rather urgent for the doctor of different specialties. The frequency of primary hypothyroidism in the population is 0,2-2,0%, and subclinial hypothyroidism is diagnosed in 10 - 12% of women and 2-3% of men [6].

Physiological effects of thyroid hormones are rather clearly verified and stipulated not only by their direct effect on the expression of genes controlling synthesis of structural and functional proteins in the cells of different body systems, but indirectly as well – through their interaction with catecholamines providing metabolic processes [1, 10, 13]. The main signs of thyroid hormones include: 1) increase of mitochondrial efficacy and myocardium contraction; 2) providing normal processes of growth, development and differentiation of tissues and organs; 3) increase of renal blood circulation, glomerular filtration and diuresis; 4) stimulation of heat production and body temperature; 5) maintenance of sexual and reproductive functions; 6) potentiation of sympathetic effects; 7) increase of excitability of the central nervous system and activation of psychic processes [3, 8, 9]. Moreover, thyroid hormones produce a considerable effect on the maturation of specific neuronal populations, therefore their lack during the period of active neurogenesis results in irreversible mental retardation and is associated with multiple morphological changes in the brain [7, 8, 12].

Undoubtedly, changes of the thyroid status and in case of hypothyroidism in particular, produce a direct effect upon the functions of practically all the body systems, and thus the mechanisms of non-specific adaptation can be involved. In its turn, it results in the development of a wide spectrum of symptoms including fatigue, weakness, weight gain and depression, memory deterioration and poor learning progress, progressive decrease of intellectual abilities, psychic disorders, ataxia, muscular weakness and

muscular convulsions, and in severe cases – coma [1, 11].

Modern scientific literature does not present sufficient information concerning the state of cognitive functions and trace elements metabolism in case of hypofunction of the thyroid gland [2, 9]. Thus, investigation of pathogenic aspects and biochemical transformations occurring against the ground of hypothyroidism stipulates a considerable interest to more comprehensive study of the issue.

Objective of the work is to find the effect of changes in trace elements content in rats with simulated hypothyroidism on the state of their cognitive functions.

Materials and methods. 42 albino male Wistar rats were used in the study with their body mass of 300-350 g. The animals were divided into two groups: I – intact; II – with experimental hypothyroidism simulated by means of everyday supplement of Mercazolil (the acting agent Thiamazole) to drinking water of animals (Ltd. Pharmaceutical company "Zdorovya", Kharkiv, Ukraine) during 30 days.

Efficacy of the suggested model of simulated hypothyroidism was evidenced by the results of examination of the hormonal status. The content of TSH (TSH ELISA, Germany), free triiodthyronine (T3) (T3 EIA KIT, USA), free thyroxin (T4) (T4 EIA KIT, USA) was determined in the blood serum of rats. The body mass of animals was estimated. In addition, reliable changes specific for hypothyroidism were detected by means of morphological examinations of the thyroid gland and published earlier [1].

The whole blood was used as the material for the study. The contents of macronutrient element magnesium (Mg) and trace elements zinc (Zn) and copper (Cu) were determined. The blood was taken for analysis 1 month later since the beginning of the experiment after decapitation performed under ketamine narcosis (40 mg/kg). The obtained 1 ml of blood was placed into the porcelain crucibles and burnt in the muffle furnace at the temperature of 450 ^{0}C during 48 hours. Then the ashes received were dissolved in 10% hydrochloric (2 ml) and nitric acids (1 ml), and brought to 10 ml adding bi-distilled water. The solution prepared was analyzed on the atomic-absorptive spectrophotometer С-115ПК according to the common methods.

To examine and assess cognitive functions the following tests were used: 1) «Open field», enabling to study the animal behavior considering the level of their motor activity (the number of squares in the cage crossed by a rat and rotation movements available), orientation-learning reactions (number of vertical positions and looking into the holes on the floor of the cage), emotional lability (by the number of defecations and urinations), grooming during 2 minutes; 2) test of «Social cognition» during which rats were kept in cages alone for a week, then a juvenile male rat was added, and the time of acquaintance till the moment of losing interest to a young guest was fixed. The latter was taken from the cage for 40 minutes and then returned back; after that the time spent for a repeated acquaintance was fixed again. 3) test to detect «New object» based on the interest of rats to learn new things. The animal was placed in an empty cage containing two similar objects, and the time spent on their learning was fixed. 3 minutes later the objects and the animal were taken away. 40 minutes later the experimental animal was placed back into the cage together with one previously examined object and a new one different by its colour and shape. The time difference in learning new and old objects was fixed.

The data obtained were processed by means of non-parametric Wilcoxon statistical criteria and Sign-test applying the program «Statistica 7» («Statsoft, Inc.» – USA). The differences were considered reliable in case P was 95% and more ($p<0,05$).

Results. After the first week of the experiment the rats with simulated hypothyroidism developed diminution of physical activity, loss of interest to the environment and satisfactory appetite. 4 weeks later the rats of II group were inert, low-activity, they demonstrate lack of interest to the surrounding factors, the signs of hair loos, in some places hair became yellowish. A part of the animals developed dermatological changes: dry skin with its desquamation, erosions and ulcers.

T3 and T4 levels in the animals from II group with hypothyroidism were found to decrease 3,9 and 3,3 times ($p<0,05$) respectively as compared to the findings of the intact animals. At the same

time, TSH in the blood serum of this group of animals increased more than twice reliably which may be indicative of the response of the hypothalamic-pituitary system and considerable decrease of thyroid hormones content.

As compared to the control the body mass in II group increased more than on 25% ($p<0,05$), which is typical for hypothyroidism, while in I group the body mass did not increase reliably.

One of the important and compulsory conditions for normal functioning of the body is stability of the chemical blood content. The blood reflects a current state of element balance in the body. Thus, the concentration of trace elements in the animals with hypothyroidism was the following: Cu increased on 42,93% ($p<0,05$), Mg – 27,84% ($p<0,05$), and Zn – remained on the level of that of the control. Similar tendency was found concerning biological elements in the raw substance. In II experimental group Cu concentration increased1,54 times as much ($p<0,05$), Mg – 23,36% ($p<0,05$), and Zn – 6,8% ($p>0,05$).

Investigation of orientation-learning and cognitive behavior in II group of animals found that the longer the period of the experiment was the more progressive inhibition of cognitive functions was marked. Thus, the number of the examined holes as a signs of hole reflex, which is indicative of the ability of animals to be examined in the open field test, was reliably lower in rats with hypothyroidism. Although the result of the experiment in open field were not only indicative of inhibition of learning functions, but registered less positions on the posterior limbs in the experimental hypothyroidism.

Changes of the character of activity directed to the identification of new subjects were determined, evidenced by the results of the social cognition and new objects tests. They helped to register reduced interest both to unknown rats and unknown objects. The time of identification of old objects was twice as much longer in comparison with that of the control. The changes indicated are the signs of disturbed organization of normal learning behavior and memory, in the situation causing anxiety in particular.

An important part of the experiment was investigation of the emotional status of the animals assessed by the number of defecations and urinations. Its increase was detected only at the beginning of the experiment which is a sign of anxiety. At the same time, the character of behavior of animals with hypothyroidism at the 3rd week of the experiment is indicative of reduced reactive behavior.

Grooming should be mentioned here as an important characteristics of hygienic behavior of animals. Usually rodents spend a lot of time scratching their hair in comparison with moving in the space which is closely correlated with their motor activity. The experimental animals of II group demonstrated pronounced inhibition of grooming and motor activity while the period of the experiment became longer.

Discussion. Increased concentration of copper and magnesium in case of hypothyroidism attracts certain attention in the analysis of the main blood trace elements. This reaction of biological elements can be explained by compensative-adaptive reactions of the body in response to the development of thyroid dysfunction resulting in the utilization increase of the examined elements in potentiated metabolic processes.

It should be noted that copper is a trace element with antioxidant properties contained in ceruloplasmin, cytochromoxidase, tyrosinase, ascorbinase and other enzymes. Copper participates in hemopoiesis and metabolism of iron, metabolism and oxidation of ascorbic acid, epinephrine, serotonin, regulation of the balance of biogenic amines in the blood, processes of myelinization in the nervous system. Thus, hypothyroidism is characterized by muscle weakness, myalgia, especially in the proximal groups; moreover, typical signs of hypothyroid myopathy – cramps and slow muscular relaxation correlate with dissociation of copper concentration found in the group of experimental animals. This element is important for the maintenance of normal structure of the connective tissue fibers, influences upon carbohydrate metabolism, and tissue respiration. In addition, copper possesses pronounced anti-inflammatory properties, decreases the severity of autoimmune diseases [13].

According to the data suggested by certain authors, the excess of such biologically active elements as Cu and Mg, able to get combined with iodine, can intensify available iodine deficiency

and result in the development of diffuse goiter [2].

Magnesium belongs to the intracellular chemical elements. Its ions participate in carbohydrate and phosphorus metabolism. It is contained in many enzymes: cholinesterase, γ-glutaminecysteanase (control of the first stage of glutathione synthesis), glutaminesynthase (transforms glutamate into glutamine). Mg provides maintenance of energetic (ATP, glucose transport) and plastic (protein synthesis in ribosomes and lipoproteid complexes) processes. It participates in the synthesis of neuromediators: acetylcholine, tyrosine, norepinephrine and neuropeptides in the brain. Its level regulates the balance of lipoproteids of different density and triglycerides [5]. It possesses vasodilatory and antispastic action, stimulates intestinal peristalsis and increases bile secretion. Increased Mg concentrations in the blood can be found in case of kidney diseases with disorders of their excretory function, hypothyroidism, diabetic acidosis and is manifested by sedative effect and respiratory inhibition. Accordingly, increased magnesium content was found in rats of II group with simulated hypothyroidism at the expense of inhibition of metabolic processes which coincides with general notion concerning the content of trace elements in case of thyroid hypofunction [3].

Zinc is known to take the second position after iron concerning its content in the human body and is contained in more than 300 enzymes including alcoholdehydrogenase, DNA- and RNA-polymerase, phosphatase, dehydrogenase, carboxipeptidase, enzymes of tryptophan synthesis, etc. Therefore, its biological role is realized by means of participation of RNA and protein in synthesis, inhibition of free radical oxidation, intensification of the processes of division and differentiation of cells and tissue reparation. Moreover, Zn participates in the formation of many links of immune response and performs immune-modeling effect (decreases the intensity of allergic signs). Zinc is contained in insulin, adrenocorticotropic, somatotropic and gonadotropic hormones are zinc-dependent. Zinc participates in the synthesis of thyroid hormones preventing node formation [5, 7].

Since zinc influences upon the growth rate, every next cellular division requires Zn-dependent enzymes of RNA-polymerase, reverse-transcriptase and thymidine kinase. In spite of the fact that the latter does not contain Zn it is very sensitive to its deficiency as compared to the mentioned above. Thymidine kinase phosphorylates desoxithymidine monophosphate before its involvement in the process of replication to DNA. Moreover, zinc is a component of nuclear receptor T_3 (Zinc fingers), which due to binding with DNA regulates gene expression and synthesis of specific proteins in the cell, with further manifestation of physiological effects of thyroid hormones. On the basis of the mentioned above a decreased Zn concentration in the experimental groups is likely to occur due to its excessive use for reparation requirements of cells. Romaniuk A.M. et al suggested in their research dealing with intake of heavy metals salts that decreased zinc concentration against accumulation of such elements as copper, iron, manganese and others can be explained by their antagonistic interaction [4].

Scientists indicate a negate effect of iodine, iron, manganese, zinc and other trace elements deficiency on the formation and course of the human cognitive functions which is extremely important for children [7, 9, 10]. Moreover, there are certain data that a leading cause of autism can be imbalance between metallothionein and metals [3, 11,12].

A number of clinical studies report about serious cognitive disorders including inability to concentrate, slow thinking, reduced memory concerning the latest events, inability to calculate and understand complicated issues in case of hypothyroidism [8]. Thus, elderly people with hypothyroidism demonstrate worse abilities to learn, visual-spatial skills and attention. In addition, serious deterioration of learning, long-term and short-term memory is found in mature rats after thyroidectomy [3], which correlates with the results of the experiment conducted.

Integrated participation of trace elements in thyroid metabolism should be taken into account. Disorders of any enzymatic link in the process of formation of the thyroid gland hormones can result in changes of chemical content of tissues which in their turn will reflect morphofunctional status of the body on the whole and its cognitive ability in particular.

Conclusions. In case of mercasolil

hypothyroidism threshold and excessive concentrations of such biological elements as copper and magnesium are found in the blood of rats. Zinc content in case of hypothyroidism is found to decrease. By means of the tests “Open field”, “Social cognition” and detection of “New object” it was determined that cognitive disorders start to develop in a week after simulation of hypothyroidism in rats. Examination of orientation-learning activity gave the evidence of a negative dynamics concerning cognitive functions and memory inhibition with increased duration of hypothyroidism which is indicative of the formation of stable cognitive deficiency.

Prospects of further studies: to investigate changes of trace elements in the blood serum in order to elaborate pathogenically substantiated schemes of treatment.

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