Advances in Biology & Earth Sciences
Vol.3, No.3, 2018, pp.234-240

AGE DETERMINES THE INTENSITY OF THYROTROPIC HORMONE PRODUCTION IN RESPONSE TO COPPER SULPHATE INTOXICATION

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Abstract. The content of thyroxin and triiodothyronine in intact old animals was 30 and 23% less compared to intact young animals. Intoxication of the organism with copper sulphate in young and old animals did not affect the content of thyroxin. However, it reduced the content of triiodothyronine by 22% only in young animals. At the same time, copper sulphate intoxication was accompanied by weight loss and a decrease in body temperature, regardless of age. Elimination of weight loss and restoration of body temperature of animals due to intoxication by the administration of the mix-factor did not affect the content of thyroid hormones. At the same time, copper sulphate intoxication was accompanied by a pronounced decrease in the content of thyrotropic hormone in both young and old animals. Administration of a mix-factor to such animals did not affect its content in young animals and significantly increased its content in the serum of older animals. There is no direct correlation between the content of thyrotropic hormone during intoxication and the use of the mix-factor.

Keywords: *copper intoxication, thyroid hormones, thyrotropic hormone, aging*.

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Received*: 18 October 2018;* ***Accepted****: 14 November 2018;* ***Published****: 11 December 2018.*

1. Introduction

It is well known that extreme environmental factors induce compensatory-adaptive reactions in the organism. The nature of such adaptive reactions depends not only on the characteristics of the factors acting on the organism, but also on the metabolic states of the organism (Selye, 1974; Larzelere & Jones, 2008). This fundamental pattern is the dependence of the adaptive response on the organisms' metabolic states. It is used in experimental investigations to characterize biological systems. Thereby, long-term investigations of xenobiotics influence and diverse environmental factors on the adaptive response of young and old organisms formed the basis of the central paradigm of modern gerontology. Its essence is as follows. The ability to adequately respond to extreme environmental factors decreases with an increase in the age of animals (Morrison & Sosnoff, 2009; Terman & Brunk, 2006). Since young and old animals are influenced by the same factor, it is concluded that their metabolic systems are different. However, it is not always possible to identify significant differences in the characteristics of the metabolic systems of young and old animals in the experiment.

Such a contradiction can be explained by the fact that individual metabolic parameters are analyzed in experimental studies. We believe that one of the approaches

to solving complex integral problems of metabolic regulation may be the analysis of the states of the central metabolic regulation systems.

As one of the central regulatory systems of metabolism in response to the action of extreme environmental factors is the hormonal system (Charmandari *et al*., 2005; Fluharty, 2002; Cameron, 1993). It is known that the sympathetic adrenal and hypothalamic-pituitary-adrenocortical system plays a key role in the formation of "primary" compensatory-adaptive reactions (Forhead & Fowden, 2014). However, thyroid hormones are the most universal regulators of metabolism. It is shown that thyroxin and triiodothyronine are involved in the regulation of growth and development, tissue differentiation, regulation of general metabolism (Mondal *et al*., 2016).

Previously, it was shown that thyroxin is an uncouple of oxidative phosphorylation and regulates thermogenesis, and hyperthyroidism is accompanied by a decrease in life expectancy. At the same time, a reduced thyroxin content in animals on a calorie-restricted diet correlated with an increase in the life expectancy of such animals by 30–40% compared with the control group (Bozhkov & Nikitchenko, 2013; Bozhkov & Nikitchenko, 2014).

Such diverse effects of thyroid hormones are due to the presence of cellular receptors for them on almost all organs and tissues in the organism. It should be noted that for thyroxin and triiodothyronine there are specific receptors, it follows from this that T_4 performs its regulatory functions, and is not only a precursor of T_3 (Baas *et al*., 1994; Saicic *et al*., 2006).

As is well known, one of the most frequently occurring toxic compounds of the environment are copper ions (Lee *et al*., 2014). Copper ions have pronounced toxicity and in the case of chronic influences they induce liver fibrosis and a number of other pathologies (Heffern *et al*., 2016). Moreover, copper ions elicited various responses in young and old animals. It was previously shown that copper ions regulate the activity of enzymes synthesizing thyroxin (Bozhkov *et al*., 2017), and hyperthyroidism was accompanied by an increase in the content of copper ions in the organism. The question of the relationship of copper toxicity and thyroid function is not completely resolved. However, in the case of Wilson's disease, which is induced by the accumulation of copper ions in the organism, there is no correlation with the function of the thyroid gland.

Due to the role of thyroid hormones in the formation of Cu - induced liver fibrosis has not been established. The available data suggest that intoxication of the body with copper ions will affect the function of the thyroid gland, which may influence the formation of liver fibrosis and the restructuring of the whole organism. Age-related features of the thyroid gland response to the action of copper ions may be of great interest, since it is known that the content of thyroxin in animals decreases with age.

2. Materials and methods

Experiments were performed on young (3 months) and old (20 months) *Wistar* male rats. Animals were divided into 3 experimental groups: the control group was under standard conditions of detention, they were administrated intraperitoneally with saline solution 3 times at intervals of 48 hours in a volume corresponding to the volume of copper sulphate administered to the experimental group. The experimental group was also kept in standard vivarium conditions and animals received three times with an interval of 48 hours between doses of copper sulphate at a dose of 1 mg / 100 g body

weight at the same time, in the morning from 8 to 9 hours before feeding. The third group received copper sulphate as well as the second group, and every day between injections, they received biologically active three times - the mix-factor, which was developed and obtained in our laboratory (Kurguzova *et al.*, 2015). In each group there were 5 animals, i.e. 15 animals per experiment.

Changes in body weight and rectal temperature were determined. At the end of the experiment, animals were euthanized under ether anaesthesia in compliance with bioethical rules (Council Directive, 1986).

To obtain the blood serum of the animals, they were decapitated under anaesthesia, the blood was collected into centrifuge tubes and after 20 min of incubation at 25°C, it was centrifuged at 1,000 g/ min at room temperature. The serum was transferred to dry tubes and the content of thyroxin (T_4), triiodothyronine (T_3) andthyrotropic hormone (TH) was determined.

The content of all hormones in the serum was determined by the method of enzyme linked immunosorbent assay. Statistical analysis were determined using the Wilcoxon-Mann-Whitney criterion and Student's t-test, using the package Statistica V.

3. Results and discussions

Multiple sequential administrations (3 times) to experimental animals of copper sulphate at a dose of 1 mg / 100 g of body weight (which was 33% of the lethal) were accompanied by a loss of body weight from day 1 to day 7. These animals restored the growth of body weight after the last administration of copper sulphate. However, these animals lagged behind the control group by 8–10% by weight even after 15 days (Fig. 1A). Copper sulphate intoxication was accompanied by a decrease in body temperature, which persisted for at least 15 days (Fig. 1B). It was previously shown that such intoxication with copper ions was accompanied by the development of liver fibrosis (Bozhkov *et al*., 2017).

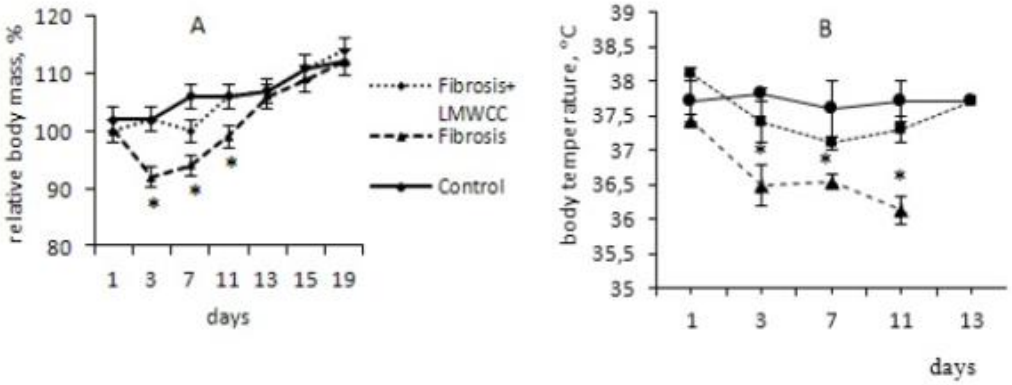

Figure 1. Changes in body weight (A) and body temperature (B) in young animals of the control group, after copper sulphate intoxication and after the administration of the mix-factor

It was shown that a decrease in thyroxin concentration in rats on a calorie-restricted diet was accompanied by a decrease in their body temperature by 1–1.5°C and an increase in their life expectancy compared with the control group of animals (Bozhkov & Nikitchenko, 2014).

It was of interest to determine the content of thyroxin and triiodothyronine in animals with low body temperature as a result of copper sulphate intoxication.

It turned out that the content of thyroxin in old animals was less by 30% compared with young animals. Triple administration of copper sulphate to animals did not affect the content of T_4, both in young and old animals (Fig. 2). It should be noted that when assessing the average values of the indicators, the individual variability of these indicators is not taken into account, and this is an important characteristic of the response. In this regard, scatter plots of thyroid hormone levels were determined.

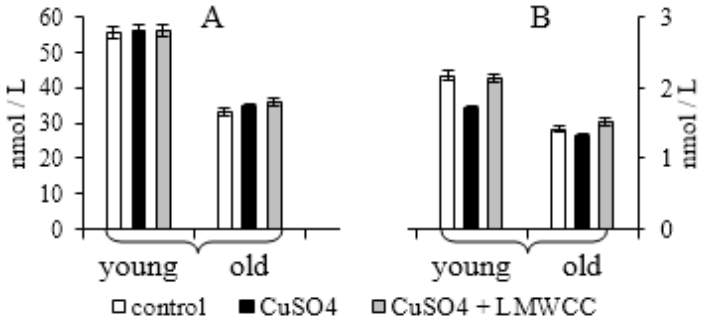

Figure 2. The content of thyroxine (A) and triiodothyronine (B) in the serum of intact animals, animals treated with copper sulphate three times at intervals of 48 hours at a dose of 1 mg / 100 g of body weight and animals that also received copper sulphate thrice received a mix-factor in a dose of 0.1 mg / 100 g body weight, in the case of young and old animals
* – the difference between the intact control and the experimental variants is noted at $P \leq 0.05$

It was found that a threefold sequential administration of copper sulphate to animals slightly increased the individual variability of the T_4 content in both young and old animals (Fig. 2).

Consequently, a decrease in the body temperature of animals against the background of intoxication with copper ions did not correlate with a decrease in their T_4 content. This can be explained by the fact that the decrease in body temperature during a calorie-restricted diet and during intoxication with copper ions is carried out by different mechanisms.

T_3 content in the group of intact old animals was reduced by 23% compared with the young (Fig. 2). Administration of copper sulphate to young and old animals was accompanied by a decrease in the T_3 content by 22% as compared with the control, while in the old it remained unchanged as compared with the control level (Fig. 2).

At the same time, the scatter plots constructed for the content of T_3 showed that in young animals the variability of the content of T_3 increased, and the response of the old animals in terms of the content of T_3 did not differ from the control values of animals of the same age.

Consequently, an increase in the content of copper ions in young and old animals did not affect the content of thyroxin, both in young and old animals. It was accompanied by a slight decrease in T_3.

We have previously found that the toxic effects of copper ions can be eliminated by the administration of low molecular weight components obtained from bovine colostrum or yeast extracts (mix-factor) (Bozhkov *et al*., 2017). In this regard, in the next series of experiments, changes in mass and body temperature in animals that were injected with a mix-factor against the background of intoxication of the organism with copper ions were determined. It was found that the administration of the mix-factor in

eliminating animal waste by body weight, and their body temperature did not differ from the control group of animals (Fig. 2).

Thyroxin content in animals that were administrated with the mix-factor against the background of intoxication did not change as compared with the control group of animals, both in young and old ones. However, if in the control variant and in the variant after the administration of copper sulphate the individual variability was not expressed, then after the administration of the mix-factor it increased, especially in the group of old animals.

Consequently, in the group of old animals, individual variability in the content of thyroxin increased after the administration of the mix-factor during intoxication.

The individual variability in the response of the thyroid gland in terms of the T_3 content in rats after the administration of the mix-factor against the background of intoxication also increased as for T_4 (Fig. 2).

Consequently, intoxication of animals with copper sulphate, which was accompanied by loss of body weight and a decrease in body temperature, did not significantly alter the content of thyroid hormones. The administration of a mixed factor to animals against the background of intoxication, which was accompanied by the normalization of body temperature and their body weight, also did not affect the functional activity of the thyroid gland. However, the mix-factor significantly increased the variability in the content of thyroid hormones, which is associated with the difference in responses in different animals, i.e. increased individual variability in the content of thyroid hormones.

As you know, one of the factors regulating the activity of the thyroid gland is thyrotropic hormone (TH), which is synthesized in the pituitary gland (Mullur *et al*., 2014). However, there is no direct correlation between the content of thyroid hormones and TH. This may be explained by the fact that hormone synthesis is regulated by a large number of various factors, in particular, the deiodinase system (Wilson, 2015). Determining the ratios of thyroid hormone and TH levels in young and old animals after copper sulphate intoxication can clarify the regulatory mechanisms of thyroid gland functioning in animal's different ages.

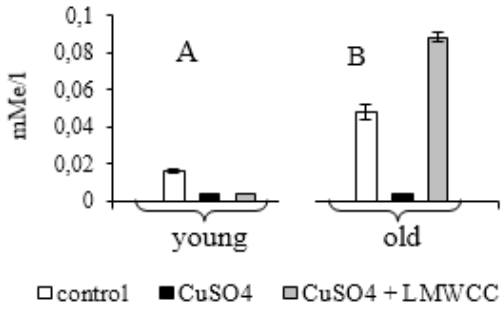

Figure 3. The content of thyroid hormone in the serum of intact animals, animals, animals treated with copper sulphate three times at intervals of 48 hours at a dose of 1 mg / 100 g of body weight and animals who also received copper sulphate, and between its injections they received a mix-factor three times at a dose of 0.1 mg / 100 g body weight, in the case of young and old animals
* – the difference between the intact control and the experimental variants is noted at $P \leq 0.05$

It turned out that the content of TH in old animals was higher than in intact young animals (Fig. 3). In the event that the animals, against the background of copper

intoxication, received a mix-factor, the content of TH in young animals remained unchanged compared with intoxication. At the same time, their content in old animals after the mix-factor increased 20 times (Fig. 3).

Consequently, copper sulphate intoxication was accompanied by a decrease in pituitary TH production and this was more pronounced in older animals, since their initial level was higher in older animals. Administration of biologically active compounds - a mix-factor on the background of intoxication did not affect the production of TH in young animals and sharply increased production in older animals.

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4. Conclusion

Results of this work allow us to draw several general conclusions:

- content of thyroid hormones decreased in the aging process;
- intoxication with copper sulphate did not affect the content of thyroxin, regardless of age, and the content of triiodothyronine decreased in young animals and did not change in older animals;
- intoxication of the body with copper sulphate was accompanied by a significant decrease in the serum TH content of young and old animals; administration of biologically active additives to animals during intoxication did not affect the TH production in young animals and significantly increased it in old animals.

Therefore, organism of old animals was more reactive with respect to the components of the mix-factor. These results are of great interest in the sense that the mix-factor can be a potential geroprotector. Our studies have shown that the mix-factor, after ingestion by animals, starting from 18-19 months of age, increased the average lifespan of such animals. These studies are still ongoing.

It is known that the production of TH by the pituitary depends on the central neurohumoral mechanisms, and copper ions, like the low molecular weight components, penetrate through the barriers and are able to influence the function of the pituitary gland.

Modern studies have shown that between TH and T_3, T_4 complex relationships are established, which depend on seasonal rhythms and individual characteristics (Kavok, 2006).

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