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Changes in No-Synthase Activity in Liver Homogenate of Rats with Hypofunction of the Thyroid Gland on the Background of Microelementosis

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Abstract.

This research highlights the study of changes in NO-synthase activity in liver homogenate of rats with hypofunction of the thyroid gland (HTG) on the background of combined iodine and copper deficiencies. Rats of both groups were kept on iodine-deficient diet for 45 days and received merkazolil with drinking water until the 15th day of the experiment. Copper deficiency was induced by daily addition of d-penicillamine to drinking water (cuprenil, 100mg/100g body weight, for 21 days). It was established that in HTG secondary to iodine deficiency the positive tendency to activation of NO-synthases in liver gomogenate was observed. The combined deficiency of microelements caused their reliable activation by 50.6-85.8%. Thus, changes in NO-synthase system in the liver were more expressed in HTG secondary to combined iodine and copper deficiencies rather then in iodine deficiency.

Keywords: hypofunction of the thyroid gland; iodine deficiency; hypoproteinemia; liver gomogenate; NO-synthases

Problem statement and the analysis of the recent research

The tendency to higher prevalence of thyroid hypofunction (TH) is observed throughout the entire territory of Ukraine. Some scientists connect this problem with insufficient attention to the role of essential microelements included in the mechanisms of thyroid hormones synthesis [7]. These include copper, but the data about its role are interpreted ambiguously. So, excessive copper intake is toxic [5]. Hepatocytes are primarily affected, because the largest accumulation of copper and the metabolism of its compounds occur in the liver [4]. Changes in functional state of the thyroid gland (TG) are known to disorganize the functioning of many systems of organism significantly, resulting in polymorphic symptomatology of thyroid pathology [7]. Disturbances in system of nitric oxide (NO) synthesis and NO-synthase activity are one of such manifestations. In recent years the important role of NO in the course of different pathologic processes in the liver has been established. Nitric ion has been proved to act as both inhibitor and agonist in mechanisms of information transmission in hepatocytes, pro- and antioxidant, inhibitor or activator of apoptosis [1]. At the same time, being powerful antioxidant NO can effectively decrease the production and concentration of free radicals in blood serum and tissues. NO-synthase (NOS) is involved in the secretion of NO during the enzymatic oxidation of amino acid L-arginine. There are three isoenzymes of NOS: Type I NOS – neuronal NOS (nNOS); Type II NOS – inducible NOS (iNOS); Type III NOS – endothelial NOS (eNOS) [6]. However, there is evidence in the literature that activation of NO-synthase may be the cause of death of macrophages, thymocytes, pancreatic cells, skeletal muscle myoblasts, neurons and some other cells of the body. Such dual action of NO-synthases does not allow us to interpret their role in the regulation of prooxidant-antioxidant balance in the tissues clearly [2].

The objective of the research was to study changes in NO-synthase activity in liver homogenate of rats with hypofunction of the thyroid gland on the background of iodine deficiency and combined iodine and copper deficiencies experimentally.

Material and methods

The research was carried out on 90 nonlinear male rats weighting 150-180 g, which were kept on iodine-deficient diet during the experiment [10]. In all animals HTG was induced by addition of thyreostatic drug mercazolilum to drinking water (7.5 mg/100g body weight) for 15 days [9]. Then, animals were divided into two groups. The rats of Group I were kept on iodine-deficient diet (TH_I, n=30). Copper deficiency was induced in animals of Group II (TH_{I+Cu}, n=30) being on iodine-deficient diet by daily addition of d-penicillamine to drinking water (cuprenil, "Polfa" Kutno Pharmaceutical Company, Poland) at a dose of 100mg/100g of body weight since the 21st till 45th days of the experiment [11]. For comparison analogical indices were examined in 30 intact animals (the control group) that were kept on standard ration, under ordinary room temperature and standard lighting conditions of vivarium. Euthanasia was performed using decapitation under ketamine anesthesia (100mg/kg body weight). Keeping, feeding and euthanasia were performed according to the international guidelines for the Humane Treatment and Management of Animals.

Hepatic copper content was determined with atomic absorption spectrophotometry using S-115 PC device (Selmi, Sumy, Ukraine) [5]. The characteristics of NO-synthase system in liver homogenate was made examining nNOS, iNOS and eNOS activities by the method of Sumbaev et al. [8]. The quantitative results of examination were made using the package of mathematical programs StatisticSoft 7.0 and Student's t-test. The difference was considered statistically significant at $p<0.05$.

Results and discussion

The development of TH was accompanied by the redistribution of copper content in the liver (Table 1). So, in rats with TH_I copper levels increased by 58.8 % ($p<0.05$) in comparison with the control group. The administration of d-penicillamine to animals caused, in turn, the decrease in copper levels by 26.6 % ($p_{1-2}<0.05$) in rats of Group II compared to analogical indices in animals with TH secondary to iodine deficiency. The reduction in hepatic copper content can affect the synthesis of copper-containing enzymes negatively, in particular ceruloplasmine, which is important component of antioxidant system of the organism.

Table 1

Hepatic copper content of intact rats, animals with thyroid hypofunction on the background of iodine deficiency, combined iodine and copper deficiencies (M±m)

	Control group (n=30)	Group I (TH_I, n=30)	Group II (TH_{I+Cu}, n=30)
Copper, mg/kg	1.87±0.40	2.97±0.20*	2.18±0.27 $p_{1-2}< 0.05$

Notes:
* - reliable difference ($p<0.05$) compared to analogical data in animals of the control group;
p with Arabic numerals - reliable difference between the indices of correspondent research groups.

Trace element deficiencies caused changes in NO-synthases activity in liver homogenate. So, in animals with iodine deficiency the positive tendency to the increase in activity of all examined NO-synthases in comparison with reference values was observed (Table 2). At the same time, the administration of d-penicillamine lead to significant activation of NO-synthases. So, the increase in nNOS, iNOS and eNOS activities by 70.2 % ($p_{1-2}<0.001$), 85.8 % ($p_{1-2}<0.05$) and 50.6 % ($p_{1-2}<0.01$), respectively compared to those in animals with iodine deficiency was observed. It is well known, that in pathologic conditions, inflammatory and dystrophic diseases of the liver in particular iNOS expression and activity increase resulting in significant increase in NO level in the organism. There is a connection between high iNOS activity and dysfunction of most vital organs. It should be noted, that eNOS manifests protective features of NO such as vasodilatation, inhibition of aggregation, opening of K^+(ATP)-channels, regulation of coronary blood circulation and heart rate. Changes in parameters of NO-synthase system in the liver of rats with TH_{I+Cu} were significanly different from those in intact animals. So, nNOS activity increased by 93.4 % ($p<0.001$), iNOS activity increased by 2.1 times ($p<0.05$) and eNOS activity increased by 79.9 % ($p<0.01$) compared to analogical indices in control animals. Correlation analysis revealed the dependence between hepatic copper content and NO-synthases activity. Strong, reverse correlative connection between the content of microelements and nNOS ($r=-0.80$) and eNOS ($r=-0.80$) activities in liver homogenate and also direct connection of medium force and iNOS activity ($r=0.53$) were established.

Table 2

Activity of NO-synthases (neuronal – nNOS, inducible – iNOS and endothelial – eNOS) in liver homogenate of intact rats, animals with thyroid hypofunction on the background of iodine deficiency, combined iodine and copper deficiencies (M±m)

Research groups	Liver		
	nNOS (nmol/min×mg)	iNOS (nmol/min×mg)	eNOS (nmol/min×mg)
Control group (n=30)	8.14±0.79	4.72±0.37	3.34±0.30
Group I (TH_I, n=30)	9.25±0.81	5.20±0.41	3.99±0.29
Group II (TH_{I+Cu}, n=30)	$15.74±0.52^{\#\#}$ $p_{1-2}<0.001$	9.66±1.89* $p_{1-2}<0.05$	$6.01±0.48^{\#}$ $p_{1-2}<0.01$

Notes:

$^{\#}$ – $p<0.01$; $^{\#\#}$ – $p<0.001$ - reliable difference compared to analogical data in animals of the control group.

p with Arabic numerals - reliable difference between the indices of correspondent research groups.

Conclusions

Considering the obtained results we can suggest that TH causes changes in NO-synthase activity in liver homogenate. Combined iodine and copper deficiencies have a more pronounced effect on the system of nitric oxide synthesis resulting in the increase in the activity of all NO-synthases, mainly due to iNOS. It can cause violation of metabolic processes in the liver.

Prospects for further research

A more detailed study of indices of NO-synthase system in the liver in case of combined iodine and copper deficiencies as well as the ability of correction and prevention of detected changes are of scientific interest. The obtained results can supplement the pathogenic mechanisms of TH development and metabolic complications of individual organs and systems, including the liver.

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