

UDC 616.44:616.153

Nechyporuk V.M.,**Zaichko N.V.,***M.I. Pyrohov Vinnytsia National Medical University, Department of Biochemistry and General chemistry, Vinnytsia, Ukraine, nechypuruk_vm@yahoo.com***Korda M.M.***HSEI «I.Ya. Horbachevskiy State Medical University», Ternopil, Ukraine***EFFECT OF THYROID GLAND FUNCTIONAL STATE ON THE FUNCTIONING OF HOMOCYSTEINE REMETHYLATION CYCLE IN RAT ORGANS**

Abstract. *L-thyroxine and Mercazolil were used for the modeling of hyper- and hypothyroidism, which were confirmed by the content of fT_3 , fT_4 and TSH. The decrease of the S-adenosylmethionine synthetase, S-adenosylhomocysteine hydrolase and betaine-homocysteine methyltransferase activities in the liver and kidneys of animals with hypothyroidism were observed. At the same time, injection of L-thyroxine increased the activity of these enzymes in the liver and kidney tissues. Hyperthyroidism caused the decrease of homocysteine concentration in blood whereas hypothyroidism increased the level of this amino acid. It has been concluded that the affection of cardiovascular system in hypothyroidism could be due to the disorders of remethylation processes in organs and tissues.*

Keywords: *thyroid hormones, remethylation cycle, homocysteine.*

Introduction. Sulphur-containing amino acids (methionine, cysteine, homocysteine and taurine) ensure the vital processes of cells, maintain redox potential and integration of cellular systems, incapacitate delirants and free radicals, support methylation processes. Disorders of metabolism of sulphur-containing amino acids due to genetic or acquired defects of enzymes which regulate their metabolism are associated with progression of variety of pathologies including Alzheimer disease, malignant tumors, neural tube defects, kidney decease. Disorder of homocysteine (HC) metabolism is particularly topical. Increase of HC stream is a serious risk factor of progression of cardiovascular deceases such as atherosclerosis, hypertension, venous thrombosis. Blood HC level directly lines up with thickness of coronary paries and contrary lines up with endothelial-dependent blood flow within coronary arteries [6].

Metabolism regulation of sulphur-containing amino acids is carried out on the different levels including by endocrine system. Thyroid hormones are one of the key hormones which regulate all the types of metabolism in organism. It was found that in patients with

hypothyroidism increase of HC content occurred, and substitutive therapy by thyroxin improved actual indicator up to the level of healthy individuals [3]. K.M. Colleran and others, 2005 [7] showed that subclinical hyperthyroidism, evoked by introduction of methimazole, causes to decrease of blood HC level. However specific molecular mechanisms of impact of thyroid hormones on increase or decrease of blood homocysteine level remain unknown. In particular the issue of impact of thyroid gland hormones on functional state of enzymes which ensure remethylation processes in organism, from which blood HC concentration directly depends on, are uninvestigated.

Objective: experimentally investigate the impact of functional state of thyroid gland on the strength of enzymes of HC remethylation cycle within liver and kidneys and blood HC level.

Materials and methods. For the purpose of investigations 40 outbred male rats of 150-180g weight each which were being kept on the standard diet were involved. All the animals were divided into 5 groups: the 1st – control group (intact rats). This group of animals was intragastrically injected 1% starch solution; the 2nd – animals in which hyperthyroidism was

provoked (daily during 14 days intragastrically L-thyroxine on the 1% starch solution by 200 mcg/day per 1 kg of body weight was injected); the 3rd – animals in which hyperthyroidism was provoked (daily during 21 days intragastrically L-thyroxine on the 1% starch solution by 200 mcg/day per 1 kg of body weight was injected); the 4th - animals in which hypothyroidism was provoked (daily during 14 days intragastrically mercazolil on the 1% starch solution by 10 mcg/day per 1 kg of body weight was injected); the 5th – animals in which hypothyroidism was provoked (daily during 21 days intragastrically mercazolil on the 1% starch solution by 10 mcg/day per 1 kg of body weight was injected); on the 14th and 21st day animals were sacrificed by the method of cervical dislocation. For the purpose of investigations blood plasma, liver and kidney tissues were used. Investigation was being carried out in accordance with common ethical principles of experiments on animals, approved by the First National Ukrainian congress on bioethics (Kyiv, 2001), “European Convention for the protection of vertebrate animals used for experimental and other scientific purposes” (Strasbourg, 1986), other international agreements and national legislation in this field.

Liver and kidneys were perfused by cold 1,15% potassium chloride solution and homogenized upon 3000rpm within 1,15% potassium chloride environment (1:3 ratio). Homogenates were centrifuging during 30 minutes upon 1500g and +4°C and derived post-nuclear fraction was being used for detection of enzymes strength that ensured the remethylation processes in organism – S-adenosylmethionine synthetase (S-AMS), S-adenosylhomocysteine hydrolase (S-AHH), betaine-homocysteine methyltransferase (BHMT). Total HC content was being detected within blood serum.

S-AMS strength was being detected by evaluation of increase of inorganic phosphate that was originated when hydrolytic cleavage of ATP when interreacting with methionine [1], S-AHH strength was being detected by evaluation of increase of sulfhydryl groups in hydrolysis reaction of S-adenosylhomocysteine [5], BHMT

strength – by evaluation of increase of sulfhydryl groups in homocysteine-betaine reaction [2].

For demonstration of hyper- and hypothyroidism states within the blood serum the content of free thyroxine (FT4), free triiodothyronine (FT3) and thyroid-stimulating hormone (TSH) were being detected by immunoenzyme method with a use of kits of “Diagnostic Systems” Company (Russia Federation) in accordance to the user tips of the manufacturing company.

Within blood serum the total HC content was being detected by immunoenzyme method with a use of kit of «Axis-Shield» Company (Great Britain).

The results were expressed as average±SEM among 8 experiments. Variations P<0,05 were considered as statistically-valid. Statistical analysis was being carried out using standard statistical software and Student's t-test.

Results and discussion. Daily injection to the animals of 200mcg/kg of L-thyroxine during 14 and 21 days caused the state of chronic hyperthyroidism that was being evidenced by increase of blood FT4 concentration in rats from the 2nd and 3rd groups by 83% (from 11,07±0,47 to 20,23±2,10 pmol/l) and by 136% (from 11,07±0,47 to 26,12±1,85 pmol/l) respectively. Wherein the TSH concentration was adequately being decreased (on the 14th day by 56% (from 0,34±0,03 to 0,15±0,02 mmol/L), on the 21st day by 76% (from 0,34±0,03 to 0,08±0,01 mmol/L). FT3 concentration when L-thyroxine injection had only increasing tendency after both periods of experiment, however when statistical analysis variances were uncertain.

Merkazolil medication (1-methyl-2-mercaptoimidazole), that blocked peroxidase enzyme that was involved in thyronine iodation within thyroid gland to triiodo- and tetraiodothyronine and decreased thyroxine incretion, was used for inhibition of end-products of thyroid hormones. Daily injection of 10mg/kg of mercazolil in the animals during 14 days caused to decrease of FT4 content in blood serum by 38% (from 11,07±0,47 to 6,84±0,27 pmol/l), medication administration during 21 day caused to almost three times of FT4 decrease (from

11,07±0,47 to 4,25±0,42 pmol/l). Daily injection of 10mg/kg of mercazolil in the animals during 14 and 21 days caused to adequate increase of TSH level by 59% (from 0,34±0,03 to 0,54±0,05 mmol/L) and by 550% (from 0,34±0,03 to 2,21±0,16 mmol/L). At the same time blood serum FT3 level decreased by 66% on the 14th day (from 2,58±0,24 to 0,87±0,06 pmol/l) and by 74% on the 21st day (from 2,58±0,24 to 0,67±0,04 pmol/l). All the data listed above shows that with a help of L-thyroxine the condition that equal to hyperthyroidism was modeled, and evident hypothyroidism was being progressed in rats in which mercazolil was injected.

HC is metabolized through one of two ways – though remethylation or transsulfuration. In normal range HC is remethylated to methionine by two ways. The first reaction is catalyzed by B12-dependent methionine synthase enzyme for which N-5-methyltetrahydrofolate, building-up process of which is occurred in the active folate cycle, is the methyl group donor. Alternative reaction of building-up of methionine from HC is catalyzed by folate-independent BHMT enzyme that exists in liver and kidneys, for which betaine is the methyl group donor. In methylation reactions not methionine but its secondary – S-adenosylmethionine, that is built-up upon conditions of methionine-ATP interaction under the impact of S-AMS enzyme, is the direct methyl group donor. S-adenosylmethionine, losing methyl group, transforms into S-adenosylhomocysteine that under the impact of S-AHH enzyme is degraded into adenosine and HC [4].

We have determined that experimental hyperthyroidism was being accompanied by increase of enzymes capacity of remethylation cycle – BHMT, S-AMS and S-AHH. Under the impact of L-thyroxine BHMT strength was increasing within liver tissue in the both follow-up periods (from 8,65±0,50 to 11,90±0,78 nM/min*mg on the 14th day and up to 12,50±0,89 nM/min*mg on the 21st day). Within kidney tissue the similar changes were observed - enzyme strength was adequately (by 35 and 47%) increasing (from 3,33±0,17 to

4,48±0,21 nM/min*mg of protein on the 14th day and up to 4,90±0,33 nM/min*mg of protein on the 21st day). At the same time S-AMS strength was adequately increasing in the both organs only on the 21st day (within liver tissue from 5,84±0,43 to 7,85±0,48 nM/min*mg of protein, within kidney tissue from 3,04±0,27 to 4,96±0,28 nM/min*mg of protein). Also only on the 21st day of experiment the strength of the next remethylation cycle enzyme was adequately metamorphosing – S-AHH (within liver tissue by 35% - from 5,66±0,30 to 7,66±0,53 nM/min*mg of protein, within kidney tissue by 59% - from 3,83±0,36 to 6,08±0,50 nM/min*mg of protein).

At the same time, upon conditions of mercazolil administration, the capacity of remethylation cycle enzymes as within liver as within kidneys was being varied backward. In particular within the liver tissue BHMT strength decreased after passing of both periods of investigation (on the 14th day of investigation by 35% (from 8,65±0,50 to 5,60±0,43 nM/min*mg of protein), on the 21st day – by 45% (to 4,95±0,27 nM/min*mg of protein)). Within the kidney tissue under the impact of mercazolil BHMT strength decreased respectively by 25 and 47% (from 3,33±0,17 to 2,15±0,19 on the 14th day and up to 1,76±0,14 nM/min*mg of protein on the 21st day of investigation).

Hypothyroidism modeling caused an inhibition of S-AMS capacity that adequately decreased within liver on the 14th day by 27% (from 5,84±0,43 to 4,25±0,29 nM/min*mg of protein) and on the 21st day by 31% (to 4,02±0,43 nM/min*mg of protein). Mercazolil injection also caused to an inhibition of S-AMS strength within kidneys – by 33% on the 14th day (from 3,04±0,27 to 2,03±0,14 nM/min*mg of protein) and by 40% on the 21st day of investigation (to 1,82±0,26 nM/min*mg of protein).

Only long-term mercazolil injection (during 21 days) was causing to adequate inhibition of S-AHH strength within liver tissue – by 24% (from 5,66±0,30 to 4,28±0,32 nM/min*mg of protein). At the same time within kidneys as on the 14th as on the 21st day of experiment the decrease

of actual enzyme strength was observed (respectively by 39% (from $3,83 \pm 0,36$ to $2,32 \pm 0,17$ nM/min*mg of protein) and by 46% (to $2,08 \pm 0,18$ nM/min*mg of protein)).

Such course of changes in the strength of foregoing enzymes of remethylation cycle in a logical way has to cause to variations in HC content. As on the 14th, as on the 21st day after L-thyroxine injection blood serum HC level was decreasing – respectively by 19% (from $8,53 \pm 0,39$ to $6,88 \pm 0,37$ mcmole/l) and 23% (to $6,53 \pm 0,45$ mcmole/l) and, vice versa, when mercazolil administration during 14 days HC content was increasing by 98% (from $8,53 \pm 0,39$ to $16,91 \pm 1,12$ mcmole/l), and during 21 days – by 160% (to $22,20 \pm 1,39$ mcmole/l).

Conclusions. 1. Increase of blood thyroxine concentration amplifies the capacity of remethylation cycle enzymes within liver and kidneys and decreases the blood HC level, while the strengths of BHMT, S-AMS and S-AHH in organs increase depending on the duration of hypertyrosinemia.

2. Hyperthyroidism state is accompanied with the decrease of enzymes strength, which is responsible for HC remethylation, within liver and kidneys. It causes to increase of blood HC level.

3. The mechanisms listed above apparently are one of the causes of abnormality of vascular tone and tendency to boosted thrombosis in patients with the hypothyroidism state.

4. In prospect it would be expedient to investigate the method of transsulfuration of the sulphur-containing amino acids when hyper- and hypothyroidism. It would empower more fundamentally understanding the molecular mechanisms which cause to metabolism

disorders of sulphur-containing amino acids when thyroid gland abnormality.

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