VARIATIONS OF INDIVIDUAL VALUES OF THE PROTEIN METABOLISM IN SIMULATED EXPERIMENTAL HYPOTHYROIDISM, IN RELATION TO THE PHENOTYPE OF ACETYLATION

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The demand for studies of various aspects, influencing the dynamics and severity of hypothyroidism is determined by prevalence of thyroid disease, as in the Central Asia as well as in other regions of the world [3]. According to the literature, the rate of metabolism of various substances in the body, including that of thyroid hormones can be affected by individual peculiarities of the processes of biotransformation and, in particular, the processes of acetylation, which depend on predominance of either of main types of N-acetyltransferase isoenzymes in organism: N-acetyltransferase 1 (NAT1) or N-acetyltransferase 2 (NAT2) [8, 10]. The processes of acetylation, that have high activity in liver and intestines, have the leading role in the processes of biotransformation of endogenous substrates possessing amino group [7]. As is known, it is precisely these organs, where metabolism of thyroid hormones predominantly occurs, in particular, the processes of their deiodination [4, 9].

Keywords: hypothyroidism, phenotype, metabolism

At the same time, the works of various researchers demonstrate the impact of hypothyroidism on the development of pathological processes and diseases other organs and systems [6]. Hence, because the changes in various biochemical parameters of blood may serve as markers of diseases, occurring in thyroid pathology, it becomes an important task to study those changes in connection with phenotype of acetylation [1, 2].

The aim of the work was to identify differences and patterns in the dynamics of protein metabolism, in accordance with acetylation phenotype in experimental hypothyroidism.

Materials and methods of research

The experiments were carried out on 60 rats with body mass of 180–220g, which were divided into two equal by the number of animals main groups, depending on the type of acetylation: α- slow and β-fast metabolizers (acetylators). An experimental hypothyroidism was simulated in both experimental groups by introducing mercazolil at a dose of 5 mg, given over 14 days [3]. Each group in turn was divided into 5 equal subgroups, depending on the periods of study. In this way, the group of α- "slow" metabolizers (acetylators) was divided into the following subgroups: I_{α} – intact, II_{α} – 4th hour of study, III_{α} – 1st week of study, IV_{α} – 2nd week of study, V_{α} – 3rd week of study. Accordingly, the group β – "fast" metabolizers (acetylators) consisted of: I_{β} – intact, II_{β} – 4th hour of study, III_{β} – 1st week of study, IV_{β} – 2nd week of study, V_{β} – 3rd week of study. The dynamics of values of thyroid hormones, reflecting the development of pathological process, were evaluated as within the given subgroups separately, considering the rate and type of acetylation, as well as in associate $I_{\alpha\beta}$, $II_{\alpha\beta}$, $III_{\alpha\beta}$, $IV_{\alpha\beta}$ and $IV_{\alpha\beta}$ groups regardless of acetylation phenotype. The results of this comparison are shown in Table 2. Likewise, the data of two analogous subgroups, with corresponding terms but different rate of acetylation, were combined and their statistically processed results were compared to those of other combined groups with different periods of study. The comparisons are presented in Table 1, which reflect the changes of the level of thyroid hormones in groups Iαβ $I_{\alpha\beta}$, $II_{\alpha\beta}$, $III_{\alpha\beta}$, $IV_{\alpha\beta}$ and $IV_{\alpha\beta}$.

Also, the changes in the concentration of total protein and albumin were examined in the above terms and the parameters were studied as in combined groups, consisting of α and β subgroups (with phenotype of "slow" and "fast" acetylation of metabolic system of body, respectively), as well as within given subgroups separately and the reliability of differences between them were examined.

Acetylation phenotype was identified by the conventional method [1]. Biochemical parameters of concentration of substances in serum were determined by semiautomatic biochemical analyzer "Mindray BA88" (China), with test system "Human" (Germany) [5].

Statistical data processing was performed using Student's criterion using Excel and Biostat. The criterion for statistical significance was the importance of $P < 0,05$.

Results of research and their discussion

Changes of the blood biochemical parameters, characterizing intensity of anabolic processes were observed at various stages of the modelled experimental hypothyroidism.. In addition, there were changes in protein metabolism observed (Table 1). Thereby, the level of total protein in the 4th hour, the 1st, 2nd and 3rd weeks was significantly lower in respect to the index of animals from intact group by 28,1%, 40,4%, 33,5% and 54,1% respectively. Total protein concentration on the 1st and 3rd weeks of observation of experimental pathology, compared to the 4th hour, was higher with levels of 9,6% and 54,1%, respectively. Likewise, the levels of total protein had lower values on the 3rd week compared to those in the 1st and 2nd weeks, with 9,7% and 15,4% respectively.

Albumin concentration in the 4th hour and on the 3rd week of the experiment was lower relative to the values of this parameter in animals from intact group, by 17,8% and 10,4%. As compared with the 4th hour of observation, levels of albumin were significantly higher on the 1st and 2nd weeks by 16,2% and 16,8%. At the same time, with respect to the 1st and 2nd weeks of observation, albumin concentration

on the third week was significantly lower by 9,0% and 9,5%. In general, the levels of albumin had lower values in modelled experimental hypothyroidism than the levels in the intact group.

As a result of the analysis of the results obtained in groups, separated according to the acetylation phenotype, statistically significant differences were found in the studied parameters (Table 2).

Table 1

Changes of individual biochemical parameters in blood of rats with modelled hypothyroidism (M ± m)

Studied groups	Total protein (g/l)	Albumin (g/l)
$I_{\alpha\beta}$ groups of intact animals	67,50 ± 2,0	31,60 ± 0,9
$II_{\alpha\beta}$ groups (4th hour)	52,68 ± 0,74*	26,83 ± 0,60*
$III_{\alpha\beta}$ groups (1st week)	48,05 ± 0,86*^	31,17 ± 0,18^
$IV_{\alpha\beta}$ groups (2nd week)	50,53 ± 0,78*	31,33 ± 0,18^
$V_{\alpha\beta}$ groups (3rd week)	43,80 ± 0,47^#&	28,60 ± 0,48*^#&

Notes: * – significant difference (P < 0,05) when comparing results with those in the group of intact animals; ^ – same in comparing results with those in group 1; # – same in comparing results with those in group 2; & – same when comparing results with those in group 3; $ – same in comparing results with data in the same group to a subgroup of slow acetylators.

Table 2

Changes in individual blood biochemical parameters rat modeling of the experimental hypothyroidism subgroup with α- and β-acetylating phenotype (M ± M)

Groups, (term of study)	Subgroups	Total protein (g/l)	Albumin (g/l)
$I_{\alpha\beta}$, (intact)	I_{α}	68,4 ± 2,1	32,3 ± 1,0
	I_{β}	66,7 ± 1,9	31,1 ± 0,8
$II_{\alpha\beta}$, (4th hour)	II_{α}	56,10 ± 0,35*	25,33 ± 0,75*
	II_{β}	49,27 ± 0,69*$	28,33 ± 0,41*$
$III_{\alpha\beta}$, (1st week)	III_{α}	44,37 ± 0,21*^	31,00 ± 0,18^
	III_{β}	51,73 ± 0,95*$	31,33 ± 0,21^
$IV_{\alpha\beta}$, (2nd week)	IV_{α}	45,27 ± 0,09*^#	30,67 ± 0,21^
	IV_{β}	55,80 ± 0,23*^#$	32,00 ± 0,01^#$
$V_{\alpha\beta}$, (3rd week)	V_{α}	45,27 ± 0,09*^#	30,67 ± 0,21^
	V_{β}	42,33 ± 0,61*^#&$	26,53 ± 0,24*^#&$

Notes: * – significant difference (P < 0,05) when comparing results with those in the group of intact animals; ^ – same when comparing results with those in group 1; # – same when comparing results with those in group 2; & – same when comparing the results with those in group 3; $ – same when comparing results with the data in the same group with α-subgroup of unstable metabolizers.

In the 4th hour, the 1st, 2nd and 3rd weeks the level of total serum protein was lower as compared with the values of animals in intact group in the subgroups of α-acetylation phenotype by 21,9%, 54,2% 51,1 and 51,1% respectively, and was lower by 35,4%, 28,9%, 19,5% and 57,6% respectively in animals with β- phenotype of metabolism. From obtained results it is clear that the differences in early terms (in the 4th hour) are more pronounced among subgroups with β-acetylation phenotype, but more pronounced differences at later terms, which invariably remain at around the same values in respect to intact animals, were observed among subgroups with α- slow phenotype of metabolism. At the same time, in the animals with β- phenotype of metabolism the changes relatively to I_{β} subgroup are characterized by greater lability and the difference in values of the 1st and 2nd weeks is less significant, which becomes more obvious when compared with values of animals with α – "slow" type of metabolism, significantly increasing only at latest terms, reaching and even slightly exceeding the analogous difference in subgroups with α-acetylation phenotype. In comparison with II_{α} subgroup, lower values of total protein were observed in subgroups with α-acetylation phenotype on the 1st, 2nd, and 3rd weeks by 26,4%, 23,9% and 23,9%, respectively. Concerning II_{β} subgroup, in subgroups with the according type of metabolism, there were no significant

changes observed in total protein level in the 1st week, an increase by 13,3% in the 2nd and reduction by 16,4% in 3rd week. Comparing III_{α} subgroup, in the subgroups with respective type of metabolism, lower by 2,0% levels of total protein were observed as on the 2nd as well as on the 3rd weeks of experiment, and as compared to subgroup III_{β} the levels were higher by 7,8% and lower by 22,2%, respectively. When comparing groups with α and β phenotypes of acetylation, statistically significant changes of the 2nd and 3rd weeks of study could be observed only in subgroup V_{β}, where the level of total protein was 31,8% lower than in IV_{β} subgroup.

When comparing subgroups of same terms but different rate of metabolism, in subgroups with β-acetylation phenotype relatively to α – "slow" metabolizers, there were a decrease of 13.9%, an increase of 16,6%, 23,3% and 6.9% reduction in total protein levels at 4 hour, 1st, 2nd and 3rd week terms, respectively. There were no significant differences found when comparing the total protein of intact animals with different types of metabolism.

The dynamics of the changes in the concentration of total protein had some similarity, in terms of the presence of statistically significant differences, the number of which grew with terms, but the most marked differences were seen in the ultimate terms of experiment. In line with this, significant differences in the total protein concentrations, respectively to the 4 hour study, in subgroups with β – "fast" type of metabolism appeared at later terms than in animals with α-phenotype, but more marked differences at the latest term were observed in the subgroup with β- acetylation phenotype.

Compared to the values of intact animals, the level of total protein in the subgroups with α- and β-acetylation phenotype was lower as in the early as well as in later terms. However, while at 4 hour period and 3 week period of experiment the value of this parameter was higher in the subgroup with α-acetylation phenotype than in animals with β-phenotype, the situation was quite different at the 1st and 2nd weeks.

Common ways of transformation of amino acids in the liver include deamination, transamination, decarboxylation and biosynthesis of amino acids. Changes in the concentration of total protein may indicate that one or more of the above ways of protein metabolism are impaired [4].

In the subgroups with α-acetylation phenotype, when compared with the values of intact animals, a statistically significant reduction in albumin level of 27,5% was observed at 4 hour period, however there were no significant changes observed on the 1st, 2nd and 3rd weeks. The concentration of albumin in subgroups with β – "fast" acetylation phenotype was lower by 9,8% on the 4th hour of the experiment and by 17,2% on the 3rd week. When compared with the values of the 4th hour period in subgroups with α-acetylation phenotype, levels of albumin at the 1st, 2nd and 3rd weeks were higher by 22,4%, 21,1% and 21,1%, respectively, and were higher by 10,6%, 13,0% and lower by 6,8%, respectively in subgroups with β – "fast" acetylation phenotype, examined by analogy within same periods of the experiment. When compared with the 1st week of the study, the concentration of albumin in the subgroups of animals with α- phenotype of metabolism did not significantly change at 2nd and 3rd weeks and in the groups with β-acetylation phenotype, relatively to the same periods, were higher by 2,1% and lower by 18,1%, respectively. In relation to the 2nd week of the experiment, on the 3rd week of study in subgroups with β-acetylation phenotype there was a decrease by 20,6% while in subgroups with α-acetylation phenotype within same periods of study there were no significant changes.

Statistically significant differences of albumin concentration were observed when comparing the subgroups with different acetylation phenotype within same term. Thereby, in subgroups with β – "fast" acetylation phenotype with respect to subgroups with α – "slow" type of metabolism the albumin concentration was higher by 11,8% and 4,3% at 4 hour and 2 week periods and lower by 15,6% on the 3rd week. There were no significant changes observed between subgroups of intact animals and of the 1 week of the experiment.

A considerable similarity of differences between the various terms can be noticed in studying the dynamics of changes of albumin concentration at 4 hour and 1 week of study, which occur in subgroups with different acetylation phenotype. Only the 2nd and 3rd weeks of the experiment reveal a high evidence of the changes and a number of statistically significant differences in the subgroups with β-acetylation phenotype, which become more obvious through making comparison with earlier periods. In general, the values of albumin were lower at all stages of study, in relation to those of intact animals, reaching the lowest values at early terms (4 hour term) of experimental hypothyroidism in the subgroup with α – "slow" type of metabolism, and at latest terms (3week) in the subgroup with β – "fast" subtype of acetylation.

As is generally known, albumin is not only an important component of protein metabolism, but is also interconnected with metabolism of lipids, in particular, the transport of fatty acids, which once again shows the importance of studying this indicator [4, 9].

Thus, when studying individual parameters that characterize the protein-synthetic function of the liver, it can be concluded that there are statistically significant differences in the dynamics of changes of studied parameters between the subgroups with α- and β-acetylation phenotypes, with more marked changes occurring in the subgroups with β – "fast" phenotype of metabolism. It should also be noted that significant differences of studied parameters, at comparing different terms of study, as well as making comparison among subgroups of same term, occur in the later stages of the modelled experimental hypothyroidism.

Conclusion

1. Indicators, representing intensity of the protein-synthetic function of liver and activity of anabolic processes of body in experimental hypothyroidism, are characterized in general by a decrease of parameters, compared to values of intact animals.

2. It is characteristic for the animals with β-acetylation phenotype to have more marked changes in the total protein and albumin than in subgroups with α- phenotype of metabolism at late terms of experimental hypothyroidism.

3. In groups with α-acetylation phenotype in the early stages of experiment, there is a considerable number of statistically significant changes in the concentration of total protein and albumin observed than in subgroups with β-acetylation phenotype. However, subsequent dynamics of these changes is characterized by greater stability.

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