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Age-Related Changes of Protein- and RNA-Synthetic Processes in Experimental Hyper- and Hypothyroidism

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Abstract—The rate of liver and plasma protein synthesis and the activity of liver RNA polymerases 1 and 2 were investigated in rats of various age under experimental hyper- and hypothyroidism. The rate of plasma protein synthesis decreased with age more dramatically than that of liver proteins. Hyper- and hypothyroidism exerted opposite effects on protein synthesis in rats: stimulation and inhibition, respectively. The manifestation of these effects was age related. The thyroid status of animals also influenced the balance of protein synthesis. Thyroxin administration caused preferential incorporation of a label into blood plasma proteins. Changes of thyroid status of old animals insignificantly affected the absolute values of the label incorporation into proteins and the ratio of the label incorporation into local and secreted liver proteins. Age-related decrease of total hepatic nuclear RNA-polymerase activity was due to reduction of the template-bound functionally active forms of RNA-polymerases 1 and 2. Administration of thyroxin caused initial redistribution of the enzyme activity between template-bound and free fractions accompanied by the increase of template bound RNA-polymerases. Prolonged hormonal stimulus also caused an increase of free RNA-polymerases, which reflects the increased synthesis of these enzymes. Mecrazolyl administration reduced the activity of RNA-polymerase 1 and 2. All age groups were characterized by preferential reduction of the bound form. RNA-polymerase 2 activity decreased to a greater extent than that of RNA-polymerase 1. The data suggest age-determined reactions of the body to altered thyroid status.

Key words: protein synthesis, RNA-polymerase, hyperthyroidism, hypothyroidism

Numerous effects of thyroid hormones are realized via changes in protein synthesis. According to modern conceptions, thyroid hormone interacts with its receptor and this hormone–receptor complex stimulates transcription and RNA transport from nucleus to cytoplasm [1-4]. Besides this stimulation of gene activity, thyroid hormones can directly influence the rate of polypeptide chain elongation [5, 6].

Impairments of thyroid gland function are common. Stress, environmental factors, nutritional imbalance, various diseases (e.g., diabetes, renal and coronary insufficiency) are accompanied by changes in thyroid status [7-10]. It is clear that these changes may have different metabolic consequences in young and old persons.

In the present study, we investigated the responsiveness of the protein synthesizing system to excess and deficit of thyroid hormones, activity of RNA-polymerase 1 and 2, and the rate of liver and plasma protein synthesis during modeling of hypo- and hyperthyroidism in rats of various age.

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MATERIALS AND METHODS

One, three, twelve, and twenty four-month-old male Wistar rats (n = 178) were used in the experiments. The effect of thyroxin (Reanal, Hungary) administration on protein synthesis was studied one day after administration of a single dose (2 mg/kg), whereas activity of RNA-polymerases 1 and 2 was investigated after treatment with thyroxin for 1, 7, and 21 days. Hypothyroidism was induced by Mecrazolyl (10 mg/kg) administration up to the registration of 40-50% reduction of thyroid hormone levels in each group of animals. The time required for this decrease varied from 14 days in young (1-month-old) to 9 days in old (24-month-old) rats. The concentration of thyroid hormones was determined using kits for radioimmunoassay (Belarus Academy of Sciences).

Synthesis of liver and plasma proteins was evaluated by the incorporation of U-^{14}C-hydrolyzate of *Chlorella* proteins (Chemapol, Czech Republic) which was injected intraperitoneally 45 min before analysis. The rate of protein synthesis was evaluated from the rel-

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ative specific activities of liver and plasma proteins referred to the concentration of labeled amino acids [11].

Selective activity of RNA-polymerases 1 and 2 was determined using a model system with isolated hepatic nuclei by the incorporation of [U-^{14}C]UTP (Chemapol) into RNA in the presence of various concentrations of α-amanitin (Calbiochem, USA) [12]. For determination of the activity of the free enzymes, nuclei were suspended in buffer of low ionic strength. The suspension was incubated at 37°C for 90 min. After sedimentation of the nuclei, the supernatant was dialyzed and concentrated [13]. The activity was evaluated by the RNA-polymerase reaction in the presence of actinomycin D and poly[d(A-T)] (Pharmacia, Sweden). The template-bound activity was determined by RNA-polymerase activity of the sediment after extraction with increasing concentrations of KCl [14].

RESULTS AND DISCUSSION

The age-related changes were characterized by reduced rate of the synthesis of local and secreted liver proteins and by significant decrease of RNA synthesis and activity of RNA-polymerases.

The age-related decrease of the label incorporation varied in liver and plasma proteins. Aging was accompanied by more pronounced decrease of the label incorporation into plasma proteins than in hepatic proteins. The ratio of relative specific activity of plasma proteins to that of hepatic proteins decreased from 0.53 in young (1-month-old) to 0.33 in old (24-month-old) rats (Tables 1 and 2).

Preferential registration of the labeled precursor in proteins isolated from livers of old animals may be attributed to prevention of the release of newly synthesized proteins from liver into the blood stream [15]. This may also reflect reduced synthesis of secretory proteins due to a decrease of bound polysomes responsible for synthesis of the major proportion of exported proteins [16].

Thyroxin administration stimulated the synthesis of both hepatic and plasma proteins in all age groups (Tables 1 and 2). The maximal effect was observed in young (1- and 3-month old) rats, whereas old animals exhibited modest response. The manifestation of the thyroxin effect on synthesis of liver and plasma proteins varied in different groups. In 1-, 3-, and 12-month-old animals thyroxin administration caused more pronounced increase in the activity of plasma proteins. In old rats thyroxin administration resulted in proportional increase of the label incorporation into hepatic and plasma proteins. Preferential stimulation of the synthesis of exported proteins in young animals may reflect hormone-induced shift in the ratio of free and bound ribosomes. Increase of the proportion of bound ribosomes was found after insulin administration, cortisone injection to adrenalectomized mice, and injection of growth hormone or triiodothyronine into hypophysectomized rats [17].

Mecrazolyl administration attenuated biosynthesis of hepatic and plasma proteins. As in the case of thyroxin administration, the magnitude and age-related changes were different for hepatic proteins and plasma proteins.

There was a comparable decrease of relative specific activity of hepatic proteins in 1-12-month-old animals, whereas no significant changes of this parameter were found in 24-month-old animals. This explains less pronounced age-related differences in the specific activity of hepatic proteins in hypothyroid rats than in intact animals.

Table 1. Effect of thyroxin and Mecrazolyl on the relative specific activity of hepatic proteins in rats of various age

Age, months	Control	Thyroxin administration	Mecrazolyl administration
1	31.7 ± 2.1	49.7 ± 4.0*	25.4 ± 1.8*
3	28.7 ± 1.9	41.5 ± 2.8**	23.0 ± 1.7*
12	27.2 ± 1.7	39.9 ± 2.6*	22.6 ± 1.8*
24	21.2 ± 1.9	25.6 ± 1.9*	19.2 ± 1.4
	$p_{1-12} > 0.05$ $p_{1-24} < 0.05$	$p_{1-12} < 0.05$ $p_{1-24} < 0.01$	$p_{1-12} > 0.05$ $p_{1-24} < 0.05$

* $p_{\text{control-experiment}} < 0.05$.
** $p_{\text{control-experiment}} < 0.01$.

Table 2. Effect of thyroxin and Mecrazolyl on the relative specific activity of plasma proteins in rats of various age

Age, months	Control	Thyroxin administration	Mecrazolyl administration
1	15.5 ± 1.1	26.7 ± 2.0**	9.7 ± 0.8**
3	12.1 ± 0.8	21.5 ± 1.4*	8.3 ± 0.7**
12	8.5 ± 0.7	13.0 ± 1.0*	6.2 ± 0.4*
24	7.1 ± 0.4	8.7 ± 0.5*	5.0 ± 0.3*
	$p_{1-12} < 0.01$ $p_{1-24} < 0.01$	$p_{1-12} < 0.01$ $p_{1-24} < 0.01$	$p_{1-12} < 0.05$ $p_{1-24} < 0.01$

* $p_{\text{control-experiment}} < 0.05$.
** $p_{\text{control-experiment}} < 0.01$.

In contrast to hepatic proteins, Mecrazolyl-induced decrease of plasma proteins was maximal in young (1-3-month-old) rats. Old animals were characterized by insignificant decrease of the label incorporation into plasma proteins. However even under hypothyroid conditions there were clear age-related differences in the intensity of synthesis of plasma proteins. The maximal relative activity of plasma proteins was found in 1-month-old rats and then this parameter gradually decreased up to the age of 24-month-old.

Thus, the development of hypothyroidism influenced the balance of protein synthesis in the liver and caused preferential decrease of biosynthesis of secreted proteins. This is consistent with a report from another laboratory [18]. Hypothyroid animals were also characterized by smoothed age-related differences between parameters of synthesis of hepatic and plasma proteins. This smoothening was more pronounced in the case of hepatic proteins. The latter may be due to some differences in age-related synthesis of local hepatic proteins and secreted proteins.

The effect of thyroid hormones on the rate of protein synthesis is realized via their effects of the transcription processes. Thyroid hormones activate expression of a limited number of genes [3, 19]. They are potent regulators of transcription processes; however, their role in the regulation of transcription processes in various age groups requires further investigation. Thyroid hormones cause an increase of template activity of chromatin and rate of mRNA and protein syntheses [3, 4, 19]. The effect of thyroid hormones on transcription is determined by their interaction with thyroxin responsive DNA element resulting in increase of the number of transcription sites [4, 20, 21]. The rate of RNA synthesis is also determined by RNA-polymerase activity. The intensity of these processes may be evaluated by the level of RNA and the ratio of free and bound fractions of RNA-polymerase.

In the cell RNA-polymerases form two pools: template-bound enzymes responsible for RNA synthesis and free enzymes representing an enzymatic "reserve" that refills the fraction of template-bound enzymes to reimburse enzymes inactivated during catalysis.

Study of free and template-bound hepatic RNA-polymerases 1 and 2 revealed that aging is accompanied by a decrease of the total nuclear RNA-polymerase activity due to reduction of the bound forms of RNA-polymerase 1 and 2 (Tables 3 and 4). The activity of free RNA-polymerases reached its maximum in 3-month-old rats and remained at the same level up to 24 months. This is consistent with previous observation from another laboratory [14]. This suggests that age-related decrease of RNA-polymerase activity may be attributed to enzyme elimination from the actively

Table 3. Effect of thyroxin on the activity of liver RNA-polymerase 1 (pmol [U-^{14}C]UTP per mg DNA) in rats of various age

Experimental conditions	Form of RNA-polymerase	Age, months		
		1	3	24
Control	bound	325.7 ± 14.6	273.5 ± 36.2 $p_{1-3} > 0.05$	174.6 ± 14.0 $p_{3-24} < 0.05$
	free	217.1 ± 28.4	327.4 ± 41.6 $p_{1-3} < 0.05$	279.8 ± 31.2 $p_{3-24} > 0.05$
Thyroxin administration for:				
1 day	bound	341.9 ± 19.2	392.6 ± 12.4* $p_{1-3} < 0.05$	181.7 ± 12.2 $p_{3-24} < 0.01$
	free	297.8 ± 40.4	212.8 ± 21.2* $p_{1-3} < 0.05$	317.2 ± 17.8 $p_{3-24} < 0.05$
7 days	bound	391.7 ± 31.4*	438.2 ± 41.3** $p_{1-3} > 0.05$	217.4 ± 20.2 $p_{3-24} < 0.05$
	free	322.3 ± 29.8**	164.2 ± 31.7** $p_{1-3} < 0.01$	381.3 ± 21.6* $p_{3-24} < 0.01$
21 days	bound	387.8 ± 21.7*	451.3 ± 31.0** $p_{1-3} < 0.05$	221.8 ± 19.7** $p_{3-24} < 0.01$
	free	328.4 ± 31.2*	178.4 ± 43.6** $p_{1-3} < 0.05$	377.4 ± 32.5** $p_{3-24} < 0.01$

* $p_{\text{control-experiment}} < 0.05$.
** $p_{\text{control-experiment}} < 0.01$.

Table 4. Effect of thyroxin on the activity of liver RNA-polymerase 2 (pmol [U-^{14}C]UTP per mg DNA) in rats of various age

Experimental conditions	Form of RNA-polymerase	Age, months		
		1	3	24
Control	bound	217.3 ± 14.9	189.6 ± 20.5 $p_{1-3} > 0.05$	91.4 ± 17.3 $p_{3-24} < 0.01$
	free	111.2 ± 23.7	179.7 ± 19.1 $p_{1-3} < 0.05$	171.8 ± 36.1 $p_{3-24} > 0.05$
Thyroxin administration for:				
1 day	bound	221.4 ± 17.4	221.2 ± 29.1 $p_{1-3} > 0.05$	90.2 ± 18.9 $p_{3-24} < 0.01$
	free	102.6 ± 14.0	192.4 ± 23.1 $p_{1-3} < 0.05$	180.2 ± 37.3 $p_{3-24} > 0.05$
7 days	bound	297.6 ± 14.3*	310.3 ± 17.8** $p_{1-3} > 0.05$	97.7 ± 14.1 $p_{3-24} < 0.01$
	free	37.1 ± 16.2**	226.8 ± 17.2* $p_{1-3} < 0.01$	180.2 ± 37.4 $p_{3-24} > 0.05$
21 days	bound	307.1 ± 18.9*	318.2 ± 21.9** $p_{1-3} > 0.05$	134.4 ± 19.1 $p_{3-24} < 0.01$
	free	49.8 ± 19.7**	231.2 ± 19.4* $p_{1-3} < 0.01$	201.8 ± 6.4 $p_{3-24} > 0.05$

* $p_{\text{control-experiment}} < 0.05$.
** $p_{\text{control-experiment}} < 0.01$.

Table 5. Effect of Mecrazolyl on the activity of liver RNA-polymerase 1 (pmol [U-^{14}C]UTP per mg DNA) in rats of various age

Experimental conditions	Form of RNA-polymerase	Age, months		
		1	3	24
Control	bound	346.2 ± 15.1	284.8 ± 21.3 $p_{1-3} < 0.05$	150.5 ± 12.6 $p_{3-24} < 0.01$
	free	231.6 ± 21.0	330.2 ± 28.4 $p_{1-3} < 0.05$	263.4 ± 19.8 $p_{3-24} < 0.05$
	total	549.3 ± 48.2	619.1 ± 52.5 $p_{1-3} > 0.05$	392.7 ± 22.1 $p_{3-24} < 0.05$
Hypothyroidism	bound	258.9 ± 18.4*	193.7 ± 14.3* $p_{1-3} < 0.05$	120.4 ± 8.3* $p_{3-24} < 0.05$
	free	195.2 ± 11.6	251.0 ± 19.6* $p_{1-3} < 0.05$	221.3 ± 14.2* $p_{3-24} > 0.05$
	total	398.5 ± 24.8*	428.9 ± 31.9* $p_{1-3} > 0.05$	315.6 ± 22.4* $p_{3-24} < 0.05$

* $p_{\text{control-experiment}} < 0.05$.

Table 6. Effect of Mecrazolyl on the activity of liver RNA-polymerase 2 (pmol [U-^{14}C]UTP per mg DNA) in rats of various age

Experimental conditions	Form of RNA-polymerase	Age, months		
		1	3	24
Control	bound	233.5 ± 17.6	196.8 ± 23.2 $p_{1-3} > 0.05$	88.6 ± 15.3 $p_{3-24} < 0.05$
	free	128.0 ± 11.6	202.5 ± 18.8 $p_{1-3} < 0.05$	169.4 ± 19.3 $p_{3-24} > 0.05$
	total	356.1 ± 22.4	389.7 ± 22.6 $p_{1-3} > 0.05$	239.3 ± 17.4 $p_{3-24} < 0.01$
Hypothyroidism	bound	142.4 ± 8.2*	124.0 ± 9.2* $p_{1-3} > 0.05$	57.6 ± 3.8* $p_{3-24} < 0.01$
	free	101.2 ± 7.4*	164.3 ± 11.7* $p_{1-3} < 0.05$	135.5 ± 9.9* $p_{3-24} < 0.05$
	total	220.6 ± 14.9*	285.2 ± 22.9* $p_{1-3} < 0.05$	153.7 ± 17.4* $p_{3-24} < 0.01$

* $p_{\text{control-experiment}} < 0.05$.

functioning pool rather than absolute deficit of RNA polymerase.

Thyroxin administration caused an increase of template-bound RNA-polymerase 1 only in the group of 3-month-old rats, whereas augmentation of free enzyme was observed in 1 and 24-month-old rats. An increase of bound RNA-polymerase 2 was also observed only in the group of 3-month-old rats, but an increase of free enzyme was not found in any age groups (Table 4).

Mecrazolyl administration decreased RNA-polymerase activity in liver nuclei (Tables 5 and 6). Although RNA-polymerase activity decreased in both bound and free fractions, more pronounced changes were found for the bound enzyme. The latter suggests not only narrower range of transcription but also a decrease of the enzyme content and/or reduction of their turnover in RNA synthesis.

Age-related behavior of RNA-polymerases in hypothyroidism has some characteristic features. The lowest activity of RNA-polymerase 1 was found in 3-month-old rats. Older animals are more tolerant to the development of hypothyroidism.

The activity of RNA-polymerase 2 was more sensitive to hypothyroidism than that of RNA-polymerase 1. In contrast to the latter, the decrease of RNA-polymerase 2 activity did not exhibit any age-dependence at least within the range of 1-24 months. This might suggest comparable levels of thyroxin-sensitive transcription within these age groups.

Hypothyroidism and aging are characterized by reduced RNA synthesis at relatively high level of RNA-polymerase. This suggests that the decrease of RNA synthesis in the liver may be explained by reduced availability of DNA as the template rather than deficit of RNA-polymerases. In aging, the decrease of synthesis of RNA-polymerases is less pronounced than that of total proteins. This may be explained by multiple localization of RNA-polymerase genes [22].

Thus, the present study has revealed age-dependence of the effect of excess and deficit of thyroid hormones on protein synthesis in the liver and activities of RNA-polymerases 1 and 2. Young animals are more susceptible to the changes in thyroid status. Old animals are characterized by formation of a special metabolic state that is rather resistant to fluctuations in the level of endocrine regulators.

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