BIOGERONTOLOGY

Effects of Aging and Life-Prolonging Diet on Thyroid Regulation of Protein Synthesis

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The effect of thyroxin on the intensity of protein synthesis in rats of different age was studied during natural aging and in rats maintained on a low-caloric diet inhibiting aging. The intensity of protein synthesis decreased and the reaction to hormonal stimulus was absent in animals fed life-prolonging diet.

Key Words: *aging; low-caloric diet; thyroxin; protein synthesis*

Starting from the 1930s, when the possibility of inhibiting the tempo of aging in experimental animals by decreasing the caloric value of rations was shown [6] and up to the present time the use of low-caloric diet (LCD) remains the most effective method for life prolongation in experimental animals [3]. It is known that this phenomenon is based on prevention of many age-associated injuries to organs and tissues and delayed emergence or progress of numerous age-associated diseases, including tumors. Animals receiving LCD are more resistant to various unfavorable factors, *e.g.* surgical intervention, inflammatory agents, increased environmental temperature, toxins [4]. On the other hand, limited nutrition decreases the resistance to exposures requiring more active metabolism, for example cold stress, which attests to shifts in the thyroid axis of metabolic regulation [7]. However the regulatory effects of thyroid hormones in rats with prolonged life were never studied before, and we therefore investigated the relationship between thyroxin (T_4) and intensity of protein synthesis during aging of these animals.

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MATERIALS AND METHODS

The study was carried out on male Wistar rats (n=70) aged 1, 3, 12, and 24 months. The rats were divided into 2 groups receiving different rations: 1) standard laboratory ration (controls) and 2) LCD (experiment). Caloric value of the ration was decreased by reducing the content of carbohydrates and lipids. The deficit of protein and bioactive substances was compensated with casein, Mendel—Osborn salt mixture, and vitamin complex [2]. The animals started receiving LCD at the age of 1 month. Twice a week the rats were weighed and their weight was maintained at a constant level (by variations in the ration) for 100 days. At the end of each 100-day period food limitations were reduced and the animals were allowed to gain 10 g, after which they again received LCD. The caloric value of the ration varied from 30 to 70% of normal ration, depending on the time course of weight gain. The use of LCD prolonged the mean life span of experimental animals by 50% (maximally 2-fold).

L-Thyroxin (Reanal) was used to study the effects of T_4 on the intensity of protein synthesis. The hormone was injected in a single dose of 200 μg/100 g 24 h before sacrifice. The content of thyroid hormones (TH) in the serum was measured by radioimmunoassay using commercial kits (Academy of Sciences of

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Belarus'). Protein synthesis in the liver and blood plasma was evaluated by incorporation of chlorella protein [U-C^{14}] hydrolysate (Chemapol) 45 min after intraperitoneal injection. The intensity of protein synthesis was evaluated by relative specific activity of liver and plasma proteins estimated as the ratio of specific radioactivity to the concentration of labeled amino acids [12].

RESULTS

In order to evaluate the relationship between TH effects and their content in the body, T_4 and triiodothyronine (T_3) concentrations in the serum were measured after functional load (T_4 injection). In controls (Table 1) TH concentration in the blood was maximum at the age of 1 month, which is in line with high production of TH and their active release into tissues at the early stages of postnatal ontogeny [11]. The liver T_4-converting activity providing the formation of the bulk of circulating T_3 is also the highest in young rats. TH level gradually decreases with age: minimum values were observed in animals aged 24 months.

Injection of T_4 to control animals leads to an appreciable increase in serum concentrations of T_4 and T_3. The highest concentration of T_3 3-fold surpassing the baseline value, is observed in rats at the age of 1 month. In other age groups TH concentrations are lower and virtually do not differ.

In rats receiving LCD the concentrations of T_3 at 3 months and of T_4 at 3 and 12 months are lower than in controls of the same age (Table 1). This is in line with essential suppression of thyroid function at the initial stages of LCD treatment [2]. T_3 levels virtually do not differ in 12-month-old rats, while at the age of 24 months T_3 concentration in experimental rats is notably higher that in the control, while T_4 concentrations are virtually the same in both groups.

After T_4 loading the increment in T_3 and T_4 levels in experimental animals was more expressed in comparison with the controls. It is known that the increase in blood T_4 concentration induced by injection of T_4 is due to the release of exogenous hormone, while the increase in T_3 level is a result of enhanced peripheral conversion of T_4 into T_3. Fasting and low-carbohydrate diet are paralleled by decreased peripheral conversion of T_4 into T_3, which is significant for the maintenance of energy balance in the body. Higher concentration of T_3 in LCD-treated animals injected with T_4 observed in our experiments seems to be due to decelerated TH metabolism, which is confirmed by a higher level of exogenous T_4 in the blood of these rats.

The detected age-associated changes in the thyroid status of rats correlate with changes in the intensity of protein synthesis. Age-associated intensity of protein synthesis in control animals is characterized by

TABLE 1. Serum Concentrations of T_4 and T_3 in Rats of Different Age Injected with T_4 (nmol/liter, *M*±*m*)

Age, months	Before injection of T_4				After injection of T_4			
	control		experiment		control		experiment	
	T_4	T_3	T_4	T_3	T_4	T_3	T_4	T_3
1	98.6±8.1	2.54±0.19	—	—	245.3±18.0°	7.24±0.61°	—	—
3	94.9±8.7	1.21±0.08*	51.7±4.8	0.74±0.06	216.8±19.4°	2.62±0.19*°	330.6±28.2°	3.51±0.29°
12	87.3±9.2	0.76±0.06*	48.5±9.2	0.70±0.07	223.3±20.3°	2.43±0.17*°	348.2±20.3°	4.02±0.31°
24	58.8±4.6	0.63±0.05	64.0±5.0	0.89±0.07+	202.6±16.4**°	3.10±0.29*°	370.5±30.1°	4.27±0.38°

Note. *$p<0.01$, **$p<0.05$ compared to 1-month-old animals; +$p<0.05$ compared to 3-month-old animals; °compared to the level before injection of T_4.

gradual decrease of synthesis of structural and secretory proteins of the liver by the age of 24 months [1]. The decrease of the label incorporation in the liver and plasma proteins with age is expressed in different degree (Table 2). Aging is associated with more drastic decrease in label incorporation into plasma proteins in comparison with liver proteins. Predominant incorporation of labeled precursor into proteins released from the liver in old animals can be due to delayed release of newly produced proteins into the blood flow or to decreased production of secretory proteins because of age-associated decrease in the percentage of bound polysomes responsible for the synthesis of "exported" proteins.

LCD appreciably reduced the intensity of protein synthesis and decreased age-specific differences in this parameter (Table 3). It is paralleled by an increase in the relative specific activity of liver proteins, while relative specific activity of plasma proteins in 24-month-old rats decreased compared to animals aged 3 and 12 months. Similar changes in label incorporation into liver structural and secretory proteins are observed in aging control animals, which indicates a universal direction of age-specific changes in the ratio of synthesis of liver structural and "exported" proteins in both groups of animals.

Despite the absence of appreciable difference in the intensity of formation of the active form of thyroid hormone (T_3) in control and experimental animals, the reactions of the protein-producing system in cells to T_4 were different. Injection of T_4 to control animals intensified label incorporation in the liver and plasma proteins during all the studied periods of ontogeny. The maximum effect was observed in young rats aged 1 and 3 months. In old rats injection of T_4 just negligibly modulated the intensity of protein synthesis. A specific effect of T_4 on induction of synthesis of structural liver proteins and plasma proteins (the majority of which are produced by the liver) was detected. Relative specific activity of plasma proteins more intensely increased at the age of 1, 3, and 12 months, while in old rats label incorporation into liver and plasma proteins increased proportionally. Predominant stimulation of production of exported proteins during the first half of ontogeny can reflect hormone-induced shift of the ratio of bound to free ribosomes in the pool of cell ribosomes. This hypothesis is confirmed by an increase in the percentage of bound ribosomes after injections of various hormones [13].

However in animals receiving LCD injection of T_4 usually associated with an appreciable increase in serum T_3 concentration did not lead to changes in the intensity of protein synthesis in all age groups, this indicating reduced reaction capacity of the cellular protein-producing system during LCD treatment. Similarly as in old controls, in animals receiving LCD the nuclear receptors of TH (an important component in the realization of hormone effect) are involved in the process of hormone signal suppression. According to some reports [8] injection of T_3 during alimentary deprivation is paralleled by a protective reaction consisting in reduction of the number of nuclear T_3 receptors.

We conclude that the mechanisms of TH supply to tissues do not much change during aging and limi-

TABLE 2. Effect of T_4 on Relative Specific Activity of Liver Proteins in Rats of Different Age ($M \pm m$)

Age, months	Before T_4 injection		After T_4 injection	
	control	experiment	control	experiment
1	31.7±2.1	15.5±1.1	49.7±24.0^{++}	26.7±2.0^{+}
3	28.7±1.9	12.1±0.8	41.5±22.8^{+}	21.5±1.4^{+}
12	27.2±21.7	8.5±0.7*	39.9±22.6**$^{++}$	13.0±1.0**$^{++}$
24	21.2±1.9*	7.1±0.4*	25.6±21.9**$^{++}$	8.7±0.5*$^{++}$

Note. *$p<0.01$, **$p<0.05$ compared to 1-month-old animals; $^{+}p<0.01$, $^{++}p<0.05$ compared to the level before injection.

TABLE 3. Effect of T_4 on Relative Activity of Liver and Plasma Proteins in Experimental Rats of Different Age ($M \pm m$)

Age, months	Before T_4 injection		After T_4 injection	
	liver	blood	liver	blood
3	14.0±0.6	6.5±0.4	14.6±0.8	6.8±0.2
12	15.0±0.7	7.0±0.6	14.5±0.8	6.4±0.4
24	16.5±0.9*	5.6±0.4*	16.3±0.9	5.9±0.4*

Note. *$p<0.05$ compared to 3-month-old animals.

tation of the ration. On the other hand, the regulatory effect of T_4 on the intensity of protein production is markedly reduced, which seems to be due to structural and metabolic rearrangements in tissues. This decreased sensitivity of the protein-producing system of liver cells to a hormonal stimulus.

These data are very informative within the framework of discussion on the intensity of metabolism in animals receiving life-prolonging diet. Some authors discussing the results consider that the intensity of metabolism is reduced in these animals [9], others consider that the effect of limited nutrition manifests without reducing the intensity of metabolism [5], though they admit deceleration of metabolism in specific organs, tissues, and cells. We consider that analysis of certain aspects of metabolism is a more adequate approach to the solution of this problem than investigation of any structural systems of the body. Our results indicate that induction of energy consuming processes, one of which is protein production, is limited in animals receiving low-caloric diets, while the reactions responsible for the formation of macroergic compounds, such as glucose utilization or gluconeogenesis, are very rapid under these conditions [10]. Hence, adaptation to LCD consists in the formation of maximally balanced metabolism ruling out irrational energy consumption, which seems to underlie the effect of life prolongation.

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