INVESTIGATION OF THYROXINE-BINDING PROTEINS AND TISSUE IODOPROTEINS OF THE LIVER

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The thyroxine-binding ability of individual electrophoretic fractions of soluble liver proteins and the formation of tissue iodoproteins in the liver were investigated during metabolism of thyroid hormones. Experiments in vitro showed the presence of three specific thyroxine-binding proteins in the liver tissue, two in the zone of γ-globulins and the third in the zone with electrophoretic mobility of the prealbumin of the blood serum proteins. The presence of several fractions of iodoproteins as components of the soluble liver proteins formed in vivo during tissue metabolism of thyroid hormones was demonstrated.

A special place in the metabolism of thyroid hormones is occupied by their deiodination which is accompanied by the formation of tissue iodoproteins [1-3, 7, 9]. However, the role of the removal of iodine from the thyronine structure of the hormone and of the formation of tissue iodoproteins in the manifestation of the physiological activity of the thyroid hormones is not yet clear. The similarity and differences between the specific thyroxine-binding proteins of the blood serum, liver, and tissue iodoproteins likewise have not yet been investigated.

The object of the present investigation was to study the electrophoretic mobility of specific thyroxine-binding proteins of the serum and liver and to determine the content of butanol-unextractable iodine and the composition of iodoproteins in the liver tissue after administration of I^{131}-labeled thyroxine to rats.

EXPERIMENTAL METHOD

Experiments were carried out on 74 male albino rats weighing 130-160 g.

The transparent liver protein extract (in 0.25-M sucrose solution) and also the blood serum were saturated with thyroxine-I^{131} by the method of Berger et al. [4]. The proteins were then fractionated by electrophoresis on polyacrylamide gel [5]. Each protein fraction on the gel was cut out separately. The degree of incorporation of thyroxine into the fraction of soluble liver and serum proteins was determined by means of the MST-17 counter and B-4 apparatus. The iodoproteins of the liver were estimated in animals which received injections of KI solution for 6 days to inhibit the iodine-absorbing function of the thyroid gland; administration of KI was stopped 24 h before the injection of thyroxine-I^{131}. The rats were sacrificed 48 h after receiving the injection of thyroxine-I^{131} (50 μCi/100 g body weight). The liver was perfused in situ with cold 0.25-M sucrose solution and homogenized. The content of butanol-unextractable I^{131} and the iodoproteins in the homogenate were determined. The composition of the iodoproteins was investigated by electrophoresis [5]. The quantitative ratio between the individual protein components was obtained by densitometry on the MF-4 microphotometer with slight modifications. The total radioactivity and the radioactivity of each individual protein fraction were counted.

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TABLE 1. Binding of Thyroxine-I^{131} with Blood Plasma Proteins and Fractions of Soluble Liver Proteins of Rats in Vitro

Serial	Fraction	Percentage of			
		blood serum proteins (M ± m)	radioactivity of blood serum proteins	soluble liver proteins (M±m)	radioactivity of liver proteins
1	γ-Globulin	4,6±0,53	—	7,2±0,86	59,3
2	γ_1-Globulin	6,5±0,47	—	6,5±0,44	6,2
3	β_1-Lipoprotein	1,7±0,08	—	8,4±1,1	—
4	α_1-Globulin	7,0±0,55	—	9,2±1,2	—
5	α_2-Globulin	6,4±0,47	—	2,6±0,15	—
6	β_1-Globulin	8,2±0,71	—	2,6±0,15	—
7	β_2-Globulin	6,3±0,46	—	3,7±0,26	—
8	α-Globulin	10,6±1,4	—	1,6±0,05	—
9	»	4,3±0,6	—	2,8±0,17	—
10	»	0,8±0,02	24,6(TBG)	2,4±0,14	—
11	»	0,5±0,02		1,9±0,07	—
12	»	4,0±0,50		8,6±1,4	—
13	Albumin	38,6±2,7	32,4 (TBA)	7,1±1,2	—
14	Prealbumin	0,8±0,04	42,8 (TBPA)	2,9±0,14	—
15	»	—	—	19,0±1,4	—
16	»	—	—	2,1±0,12	—
17	»	—	—	5,3±0,84	—
18	»	—	—	3,9±0,36	—

Note. TBG) Thyroxine-binding globulin; TBA) thyroxine-binding albumin; TBPA) thyroxine-binding prealbumin.

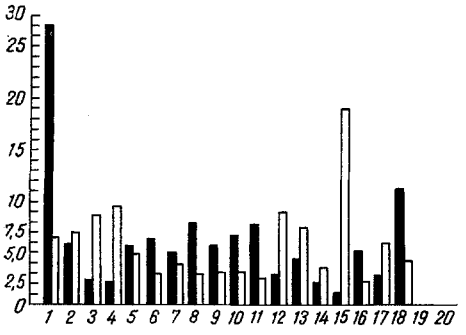

Fig. 1. Content of I^{131} in individual electrophoretic fractions of soluble liver tissue proteins. Abscissa, No. of fraction; ordinate, percentage of proteins (unshaded columns) and their radioactivity (shaded columns).

EXPERIMENTAL RESULTS AND DISCUSSION

Fractionation of the soluble liver proteins on polyacrylamide gel yielded from 18 to 20 components. After the addition of thyroxine-I^{131} to the extract radioactivity was recorded in the γ-globulin and prealbumin zones of the proteins, quite separately from the specific hormone-binding proteins of the blood (Table 1). The presence of tissue thyroxine-binding proteins has been observed by other workers [7, 11, 12].

Two fractions of thyroxine-binding liver proteins lay in the γ-globulin zone and the third fraction in the zone of the prealbumin fraction of the blood serum proteins, and they accounted for 13.7 and 3.9% respectively of the total content of soluble liver proteins. Most of the thyroxine was bound with the γ-globulin fraction.

The function of the thyroxine-binding proteins of liver tissue has not yet been fully explained. Very probably the presence of large quantities of specific thyroxine-binding proteins in the liver tissue is one of the principal factors responsible for the accumulation of a high concentration (30-50%) of exogenous thyroxine in it [2, 9]. It is also known that the cell nuclei and their extracts without cytoplasm cannot bind the hormone [6]. It can be postulated on this basis that thyroxine-binding liver tissue proteins play a role in the intracellular distribution of thyroid hormones.

The tissue iodoproteins appearing during peripheral metabolism on the thyroid hormones were investigated by determination of the content of the fraction of radioactive components and I^{131} unextractable by butanol in the individual electrophoretic fractions of soluble liver proteins after injection of labeled thyroxine. These investigations showed that as a result of metabolism of the thyroid hormones both in the structural part of the cells and in the soluble protein fraction of the liver tissue, iodinated proteins are formed. Whereas 18-25% of the total activity of the liver tissue consists of butanol-unextractable I^{131}, the butanol-unextractable activity in the soluble protein fractions was much lower and varied from 2.4 to 4%.

Results for the composition of the soluble iodoproteins of the liver and their relative percentages of radioactivity are given in Fig. 1.

As Fig. 1 shows, the greater part of the hormonal I^{131} was a component of the soluble liver proteins corresponding in their electrophoretic mobility to γ-, β-, and α-globulins and to the prealbumin fractions of the blood serum. No parallel was found between the content of the individual liver protein fractions and the level of hormonal I^{131} present in them. In addition, the iodinated proteins contained in the extractable fraction of the liver differed from each other both in the content of the hormonal I^{131} and in their electrophoretic mobility.

The results are evidence of the formation of complexes by thyroxine with several protein fractions occurring as components of the soluble liver fraction.

The formation of a complex of the hormone with cell proteins is evidently connected with the mechanism of the activity and metabolism of the hormone. The chemical composition, structure, and function of these iodoproteins have not yet been explained. However, heparin-like, antithrombin, fibrinolytic, and several other effects of iodinated peptides have been described in the literature [8]. It can be assumed that iodoproteins formed during metabolism of thyroid hormones [10, 11] play a specific role by influencing several metabolic pathways in the cell.

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