ROLE OF SPECIFIC IODOTHYRONINE-BINDING PROTEINS IN NUCLEO-CYTOPLASMIC RELATIONS OF THYROID HORMONES IN LIVER CELLS

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The role of specific iodothyronine-binding proteins of hepatocytes in the nucleo-cytoplasmic relations of thyroid hormones in rats was investigated under normal conditions, after thyroidectomy, and in experimental thyrotoxicosis. The concentration of hormone-binding sites in the cell was shown to depend on the extracellular level of thyroid hormones. The important role of cytosol hormone-binding proteins in the accumulation and intracellular distribution of thyroid hormones is emphasized. Cytosol tri-iodothyronine-binding proteins were shown to play no part in penetration of the hormone into the nucleus. The tri-iodothyronine level in the nuclei was directly dependent on the concentration of receptor proteins in them and the degree of occupancy of the acceptor sites in the receptors themselves.

KEY WORDS: thyroid hormones; liver; hormone receptors; mechanism of action of hormones.

Proteins with the function of forming specific complexes with thyroid hormones are known to be present in the cytoplasm and in various ultrastructures of the cells of target organs [2-9]. The study of the pathways of interaction between the cytosol hormone–protein complex and other components of the cell and the determination of the biological role of these interactions are of the utmost importance to the understanding of the mechanism of action of thyroid hormones.

In this investigation the role of iodothyronine-binding protein fractions in the nucleo-cytoplasmic relations of thyroid hormones was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 286 male albino rats weighing initially 120-160 g, with different body levels of thyroid hormones (thyroxine-induced hyperthyroidism or thyroidectomy). The animals were killed by exsanguination and the liver was freed from traces of blood by perfusion with ice-cold physiological saline. The liver tissue was homogenized in 4 volumes of 0.25 M sucrose, 2.4 mM KCl and 50 mM Tris-HCl, pH 7.6. The homogenate was centrifuged at 10,000g for 15 min to free it from nuclei and mitochondria. The microsome-free supernatant was obtained by centrifugation at 130,000g. The resulting cytosol was used for experiments in vitro. The nuclei were isolated from the liver homogenates by centrifugation in 2.4 M sucrose, 1 mM $MgCl_2$ [11]. A known part of the nuclei (from 0.5 g tissue) was incubated in 2 ml medium with indicator doses of thyroxine (T_4) and tri-iodothyronine (T_3) labeled with ^{125}I (specific activity 50-78 mCi/mg). The incubation medium consisted of 0.32 M sucrose, 3 mM $MgCl_2$, 0.02 M Tris-HCl, pH 7.4, and 0.03% serum albumin. In a parallel series the nuclei were incubated with labeled hormones in the presence of cytosol. The nuclei were incubated for 30 min at 4 and 37°C. After the end of incubation an equal volume of 1% Triton X-100, dissolved in 0.32 M sucrose with 3 mM $MgCl_2$, was added to the medium and it was kept on ice for 10 min. The nuclei were then isolated by centrifugation at 10,000g for 10 min and the bound radioactivity was determined. Maximal association of iodothyronines with nuclear hormone-binding sites was observed after incubation for 25-30

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TABLE 1. Effect of Cytoplasmic $^{125}T_3$-Binding Proteins on Penetration of Hormone into Nucleus and Role of Temperature

Binding of $^{125}T_3$ with nuclear fractions, counts/min per nuclei obtained from 0.5 g liver tissue	Binding of $^{125}T_3$ with nuclear fractions after addition of cytosol protein		
	quanity of cytosol protein, mg	incubation temperature, °C	counts/min per nuclei obtained from 0.5 g liver tissue
4246	0.2	37	3197
3964	0.3	37	3018
4052	0.4	37	2846
3406	0.5	4	2162
4473	0.7	4	1862
3751	0.9	4	1272
4678	1.0	4	714

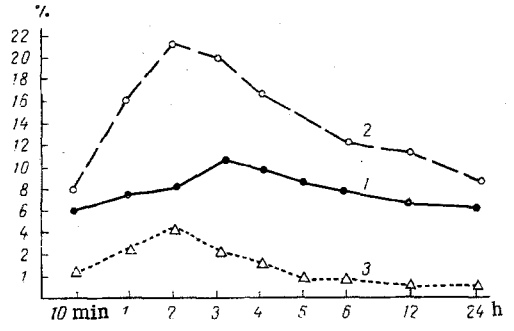

Fig. 1

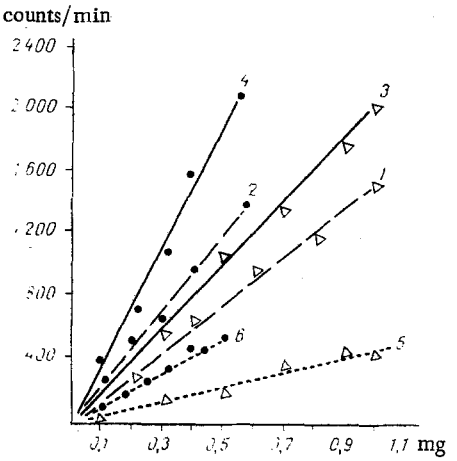

Fig. 2

Fig. 1. Dynamics of accumulation of hormonal ^{125}I in nuclei of hepatocytes of control (1), thyroidectomized (2), and hyperthyroid (3) rats after intraperitoneal injection of $^{125}T_3$. Ordinate, per cent of hormonal ^{125}I; abscissa, times of investigation.

Fig. 2. Binding of $^{125}T_3$ by proteins of nuclei and cytoplasm of liver cells of test animals. 1, 3, 5) Cytoplasm of control, thyroidectomized, and hyperthyroid rats respectively; 2, 4, 6) nuclei of control, thyroidectomized, and hyperthyroid rats respectively. Ordinate, binding of $^{125}T_3$ (in counts/min); abscissa, protein concentration (in mg).

min. The dynamics of hormone accumulation in the nuclear fraction of the liver cells was determined at various time intervals after injection of $^{125}T_3$ in vivo (50 μCi/100 g body weight). The radioactivity of the test samples was measured with an SKS-2 well-type counter.

EXPERIMENTAL RESULTS AND DISCUSSION

The results obtained by incubation of nuclei isolated from the liver of the thyroidectomized rats with radioactive iodothyronine showed that both $^{125}T_4$ and $^{125}T_3$ associate with nuclear hormone-binding sites. It is an interesting fact that the affinity of $^{125}T_3$ for nuclear fractions of the liver cells was higher than the affinity of $^{125}T_4$. The low affinity of $^{125}T_4$ for the nuclei can be explained by its lower biological activity and by the nonspecificity of the bonds which it forms.

On the addition of cytosol to the incubation medium (Table 1) binding of the iodothyronines with the nuclear fraction was not increased but, on the contrary, it was reduced. This fact is evidence that the cytosol hormone-binding proteins do not participate in the penetration of thyroid hormones to the nuclear receptors. The possibility cannot be ruled out that the hormone-binding proteins of the cytosol and nuclei differ in nature. In fact, whereas the nonhistone hormone-binding proteins are readily accepted by DNA, serum and cytoplasmic

iodothyronine-binding proteins do not associate with the nuclear components of the liver cell [7]. Furthermore, the capacity of the specific cytosol T_3-binding proteins is considerably higher than that of the nuclear receptors [10]. At the nuclear level $^{125}T_3$ is also bound by membrane proteins. This association evidently regulates the rate and extent of penetration of iodothyronine into the nuclei. It can thus be postulated that T_3, in the form of the free hormone, penetrates into the nucleus and participates in the process of modification of the receptor present in the composition of chromatin [3, 7, 8, 10].

The experiments also showed that the accumulation of $^{125}T_3$, injected in vivo, in the nuclei of the liver cells varies sharply depending on the body level of thyroid hormones. For instance, whereas about 12% of the total quantity of intracellular hormone accumulated in the nuclei of the control animals, this process took place much more intensively (23%) in thyroidectomized animals, but in the hyperthyroid rats there was a marked decrease in the accumulation of the hormone in the nuclei (Fig. 1). These facts are undisputed evidence that the concentration of hormone-binding receptors in the nucleus is limited and depends on the functional state of the thyroid gland.

In the experiments in vitro correlation was found between the specific binding of $^{125}T_3$ and the protein concentration in the incubation medium (Fig. 2). The capacity of the specific $^{125}T_3$-binding sites with high affinity for the hormones in the nucleus and cytoplasm varied in the animals with hyper- or hypothyroidism.

Much (but not all) of the hormone–protein complex, it was found, can be extracted with 0.4 M KCl. A similar fact was observed previously [1, 2, 9]. This difference in the properties of the bimolecular components at the level of the cell nucleus is evidently connected with the heterogeneity of the factors associated with thyroid hormones.

These investigations thus show that the concentration of acceptor sites for iodothyronines at the cell level varies with a change in the extracellular concentration of thyroid hormones. The accumulation and intracellular transport of thyroid hormones are controlled by specific cytosol and membrane hormone-binding proteins. The accumulation of the hormone in the nucleus depends on the concentration of specific receptor proteins and on existing unoccupied hormone-binding sites in its molecule.

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