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Specific Binding of Triiodothyronine to the Nucleoplasm

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The study of the binding of thyroid hormones to the nucleoplasm demonstrates the presence of triiodothyronine-binding molecules characterized by high affinity and limited binding capacity. The binding of thyroid analogs correlates with their thyromimetic activity. Sucrose gradient ultracentrifugation of triiodothyronine-labeled nuclear extract reveals the presence of triiodothyronine receptors, ribonucleoprotein particles with a sedimentation coefficient about 30S.

Key Words: *thyroid hormones; triiodothyronine; nucleoplasm; specific binding; ribonucleoprotein particles*

It is now well established that the effect of hormones on certain processes is realized through interaction with hormone-binding proteins which mediate the hormone signal transduction [8,9]. Classical studies postulated the existence of a single type of thyroid receptors associated with chromatin proteins acting at the level of DNA and inducing gene transcription [4,6]. However, there are numerous reports on the effects of thyroid hormones on the posttranscription processes [5,7]. These effects apparently involve hormone-binding molecules.

The aim of the present study was to reveal new hormone-binding sites in the nucleus distinct from the common (chromatin-associated) triiodothyronine (T_3) receptors by their compartmentalization.

MATERIALS AND METHODS

The nuclei were isolated from hepatocytes as described previously [11]. The nucleoplasm was isolated as follows: purified nuclei were suspended in the medium containing 10 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 0.01 M $MgCl_2$, and 5 mM β-mercaptoethanol, incubated in an ice bath for 10 min, and centrifuged. The procedure was repeated twice. The supernatants were pooled, clarified by centrifugation, and used to study T_3 binding to the nucleoplasm *in vitro*. To this end a 0.15 M NaCl extract of the nuclei (100-150 μg protein) was incubated with varied concentrations of labeled T_3 (0.125-5 nM) in the presence or absence of a 200-fold excess of cold T_3 for determination of nonspecific and total binding of the hormone. The incubation medium contained 10 mM Tris-HCl, pH 8.7, 5 mM β-mercaptoethanol, 1.5 mM EDTA, and 10% glycerin. Incubation was carried out at 0-4°C for 16-18 h. Free hormone was separated from the hormone-protein complex by filtration through Millipore membrane filters (HAWP, 0.45 μ). Nuclear ribonucleoprotein (RNP) particles were isolated as described elsewhere [2]; the animals were preliminary injected with ^{3}H-orotic acid. For evaluation of the binding of thyroid hormones to the nuclear RNP particles *in vivo* the animals were in-

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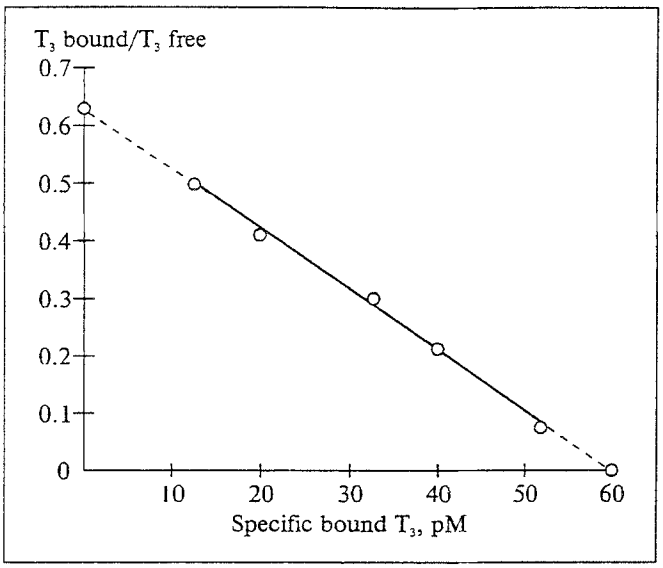

Fig. 1. Scatchard plot of ^{125}I-triiodothyronine (T_3) binding to nucleoplasm. Estimated association constant $K_{ass}=1.04\times10^{10}$ M^{-1}.

jected with ^{125}I-T_3 30 min before sacrifice, the nuclei were then isolated, and ^{125}I-labeled nuclear RNP particles were extracted.

RESULTS

Before the start of these experiments, it has been established that treatment with physiological solutions results in partial extraction of labeled T_3 bound to the nuclei. It needed to be ascertained if these T_3-binding sites comply the criteria of specific binding sites. Binding assay of the 0.15 M NaCl-extract from the nuclei with ascending concentrations of labeled hormone showed that the binding curve attained the plateau starting from a T_3 concentration of 2 nM. This attests to a limited number of T_3-binding sites in the nucleoplasm. Scatchard analysis revealed a single class of high-affinity T_3-binding molecules ($K_{ass}=1.04\times10^{10}$ M^{-1}) with the maximum binding capacity of about 60 pM (Fig. 1).

Competition binding studies of displacement of T_3 bound to the nuclear extract with an excess of unlabeled analogs (Fig. 2) showed that T_3 possesses the maximum competitive activity, thyroxin was somewhat less potent, and reverse triiodothyronine (rT_3) was the weakest competitor. This is consistent with the data on thyromimetic activity of these thyroid analogs and together with the data in Fig. 1 indicates specific binding of T_3 to the nucleoplasm.

Sucrose gradient centrifugation (15-30%) of nuclear extract revealed the presence of RNP particles with a sedimentation coefficient of 30S (Fig. 3, *2*). Gradient centrifugation of nuclear extract obtained from the liver of ^{125}I-T_3-injected rats revealed two peaks of ^{125}I-radioactivity: in a low- (~4S) and high-molecular fractions. The high-molecular peak practically coincided with the RNP peak. Spectral characteristics of the low-molecular peak ($A_{280}/A_{260}=1.09$) are indicative of proteins, while the high-molecular peak apparently represents a complex of T_3-binding proteins and RNA. Double treatment of the nuclei with 0.15 M NaCl ensured complete quantitative extraction of nucleoplasm-associated ^{125}I-radioactivity. Taking into account that chromatin (DNA-associated) T_3 and thyroxin receptors are extracted only with high salt concentrations (0.4 M and above) [4,6], the specific high-affinity T_3-binding sites of the 0.15 M

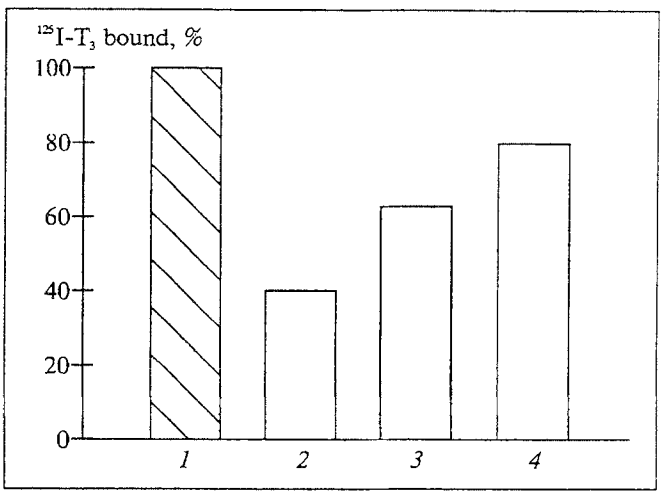

Fig. 2. Binding of ^{125}I-triiodothyronine (T_3) to nucleoplasm in the presence of unlabeled T_3 analogs. T_3 binding to the extract in the absence of unlabeled competitors taken as 100%. *1*) ^{125}I-T_3 without competitors; *2*) ^{125}I-T_3+T_3; *3*) ^{125}I-T_3+thyroxin; *4*) ^{125}I-T_3+rT_3.

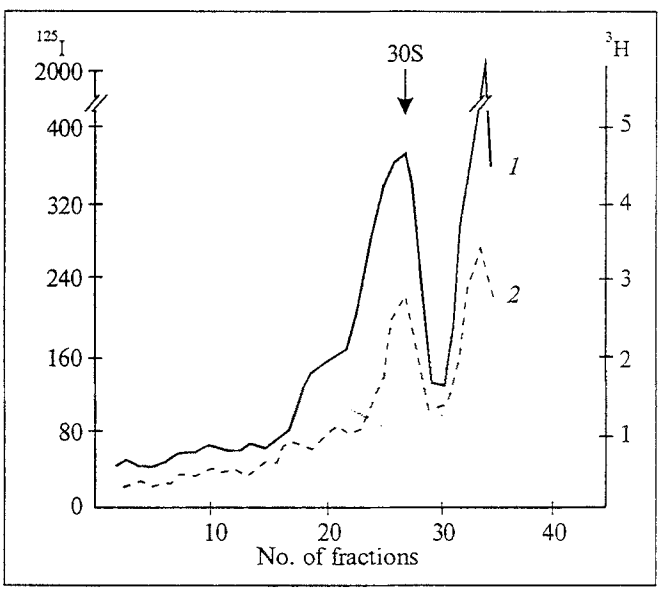

Fig. 3. Sedimentation distribution of ^{125}I-labeled nuclear extract from rat liver in 15-30% sucrose gradient. Centrifugation in an SW-41 rotor at 38,000 rpm for 4 h. Ordinate: left — ^{125}I-radioactivity, cpm (*1*), right — incorporation of ^{3}H-orotic acid, cpm$\times10^{-3}$ (*2*).

NaCl nuclear extract can be considered as an independent pool of non-chromatin-associated nuclear T_3 receptors with high affinity for RNA.

The intracellular localization of these receptor molecules is open to speculation. A. S. Spirin considers the RNP particles as a form of regulated RNA transport [10]. In line of this it can be hypothesized that the triad T_3—protein—RNA found in the nucleoplasm participates in the RNA transport from the nucleus to the cytoplasm induced by thyroid hormones. This assumption is confirmed by our previous data on the ability of nuclear thyroid receptors to stimulate the release of RNA from the nuclei in an *in vitro* transport system [1].

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