Starvation-Induced Decrease in the Maximal Binding Capacity for Triiodothyronine of the Thyroid Hormone Receptor Is Due to a Decrease in the Receptor Protein

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Biological responses to thyroid hormones are mediated by the nuclear thyroid hormone receptor (TR). Alterations in the maximal triiodothyronine (T_3)-binding capacity (Cmax) of TR measured using a ligand binding assay have been reported under some pathophysiological conditions. Northern blot analysis has indicated that TR mRNA concentrations do not necessarily correlate with Cmax levels. For example, although the decrease in Cmax in rat liver induced by prolonged fasting is well established, TR mRNA concentrations have been reported to be constant. In the present study, we examined starvation-induced changes in TR by Western blot with anti-TR(α1 + β) antiserum and by Scatchard plot analysis. Starvation of rats for 72 hours decreased Cmax in the liver to 72.5% of control levels. The 47- and 55-kd TR proteins detected in hepatic nuclear extract by Western blotting also decreased to 64% and 66% of control values, respectively. The starvation-induced changes in Cmax and TR protein levels paralleled the change in total hepatic nuclear protein concentration. These results suggest that the decrease in T_3-binding activity of the TR is due to a reduction of the TR protein itself.

THYROID HORMONE RECEPTORS (TRs) are ligand-dependent transcription factors. Tissue responsiveness to triiodothyronine (T_3) is related to TR content. Many studies have reported that the maximal T_3-binding capacity (Cmax) of TR changes under physiological and pathological conditions. Tumor growth,[1] uremia,[2] and diabetes mellitus[3] have been reported to reduce TR Cmax. In particular, a starvation-induced decrease in Cmax in rat liver has been well established. This reduction in receptor number, in combination with reduced serum T_3 concentration, is assumed to protect cells from the catabolic effects of thyroid hormone during acute caloric and amino acid deficiency.[4-7] The mechanism responsible for the starvation-induced Cmax decrease is still unknown.

There are three mRNAs encoding rat TRs with T_3-binding activity: α1, β1, and β2.[8-10] TRα1 and TRβ1 mRNAs are expressed ubiquitously in rat tissues,[11-13] whereas TRβ2 mRNA is mainly found in the pituitary.[10,14] When the amounts of individual TR mRNAs in several rat tissues were determined by Northern blot analysis in conjunction with solution hybridization, TRβ1 mRNA was the predominant receptor in the liver.[15] Lane et al[16] reported that the level of hepatic TRβ1 mRNA did not change by prolonged fasting despite a decrease in Cmax. Discrepancies between TRα1 and TRβ1 mRNA levels and Cmax have also been found in some tissues and at specific stages in development.[15,17] These results raise a question as to whether starvation-induced alterations of Cmax correlate with changes in TR protein levels.

Previously, we have measured TR protein content in rat tissues using Western blot analysis with an anti-TR antiserum that recognizes TRα1 and TRβ.[18] The 47- and 55-kd proteins were identified as TR proteins. Although discrepancies between levels of functional TR mRNA and Cmax were known to exist in rat liver and brain, we demonstrated that the relative concentration of TR proteins correlated with Cmax in both tissues.

In the present study, we measured Cmax and TR protein level by a T_3-binding assay and Western blot analysis, respectively, and examined whether the decrease in Cmax induced by starvation was due to a reduction of TR protein content.

MATERIALS AND METHODS

Preparation of Nuclear Extracts

Wistar male rats (150 g body weight) purchased from Shimizu (Kyoto, Japan) were fed a laboratory diet (Oriental Yeast Industrial, Chiba, Japan) for a few days. The manipulated group of rats were fasted for 72 hours before killing. All rats were allowed unlimited access to water. After the animals were killed by exsanguination through the abdominal aorta, the liver was immediately removed and nuclear extracts were prepared as described previously.[19] The nuclear proteins were fractionated by high-performance liquid chromatography (HPLC) using a gel-filtration column (G3000SW; Tosoh, Tokyo, Japan) in 0.05 mol/L sodium phosphate buffer, pH 7.4, with 1 mmol/L $MgCl_2$. The TR fraction was obtained by measurement of T_3-binding activity in each fraction.[18]

Protein concentration was measured using Coomassie brilliant blue G-250 (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as the standard. DNA content was determined by Burton's method.[20]

Western Blot Analysis

The procedure for raising the anti-TR antiserum, 4BII, and its characteristics have been described previously.[19,21] 4BII recognizes functional TRs (α1 and β1) but not the variant form (α2) biochemically and immunohistochemically.[19,21,22]

TR proteins were analyzed by Western blotting as described previously.[18] Briefly, proteins (20 to 60 μg per lane) in the nuclear

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Table 1. Changes in Body Weight and Total Hepatic Nuclear Protein Induced by Starvation

Group	Body Weight (g)	Nuclear Protein (mg)	
		Per Liver	Per mg DNA
Control (n = 5)	187.5 ± 14.6	7.8 ± 1.4	0.67 ± 0.04
Starved (n = 5)	133.7 ± 11.6†	5.4 ± 0.8*	0.44 ± 0.01†
	(71.3%)	(68.9%)	(65.7%)

NOTE. Values are the mean ± SD. Percent decrease is shown in parentheses.

*$P < .05$, †$P < .01$: *v* control.

extracts or the TR fraction partially purified by HPLC were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis with a 10% acrylamide gel, and then electrophoretically transferred to nitrocellulose membranes. After being blocked with Block Ace (Yukijirushi Nyugyo, Sapporo, Japan), the membrane was incubated with 4BII (1.0 μg/mL) at 4°C overnight and then with antirabbit IgG antibody conjugated with alkaline phosphatase (Tago, Burlingame, CA) at room temperature for 2 hours. The membrane was stained with Nitro Blue Tetrazolium/5-bromo-4-chloro-3-indonyl phosphate *p*-toluidine salt. Intensities of TR protein bands were measured by a laser densitometer.

T_3-Binding Assay

Nuclear extracts (50 μg protein) or the TR fraction purified by HPLC were incubated with various amounts of ^{125}I-T_3 (22.3 GBq/mg; Dainabot, Tokyo, Japan) in 5 mmol/L dithiothreitol, 0.1% bovine serum albumin, 0.4 mol/L KCl, and 0.05 mol/L Tris hydrochloride, pH 7.85, at 20°C for 3 hours or at 4°C overnight.[18] Bound and free forms of ^{125}I-T_3 were separated by adding 1 mL 2% resin (Dowex 1-X8 Resin; Bio-Rad, Richmond, CA) suspension. The nonspecific binding obtained in the presence of large amounts of nonlabeled T_3 was subtracted from the total binding. T_3-binding activity was assayed by Scatchard plot analysis, and Cmax was measured per milligram nuclear protein and per milligram DNA.

Statistical Analysis

Differences between means in control and starved groups were evaluated by ANOVA in combination with Student's *t* test.

RESULTS

Starvation of rats for 72 hours decreased body weight and total hepatic nuclear protein per milligram DNA to 71.3% and 65.7% of control values, respectively (Table 1). When T_3-binding activity in nuclear extracts was assayed by Scatchard analysis, fasting reduced the Cmax per milligram DNA to 72.5% of that in control rats, but not the Cmax per milligram nuclear protein (Table 2).

Using aliquots of the same samples used for T_3-binding assay, we examined the starvation-induced alteration of TR proteins by Western blot analysis. TR protein concentrations per milligram nuclear protein did not differ between starved and control rats. When TR protein levels were expressed per milligram DNA, starvation reduced the 47- and 55-kd TR proteins to 64% and 66% of control levels, respectively. The reduction of TR protein per milligram DNA was comparable to the decrease of Cmax per milligram DNA (Table 2).

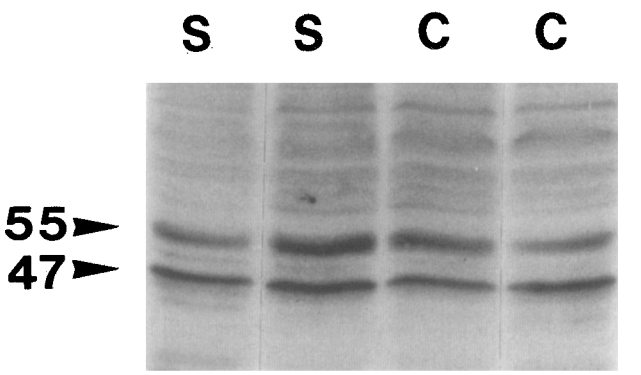

Fig 1. Western blot analysis of TR protein in starved (S) and control (C) rat liver. The TR fraction purified by HPLC (20 μg protein per lane) was probed with 4BII. The 47- and 55-kd TR protein bands are indicated at left.

To increase the accuracy of Western blot analysis, we partially purified hepatic nuclear extracts by gel-permeation HPLC. Western blot analyses of the purified TR fraction demonstrated that the relative concentrations of 47- and 55-kd proteins expressed per milligram protein in starved rats were 91% and 85% of control levels, respectively (Fig 1 and Table 3). TR proteins by Western blot expressed per milligram nuclear protein partially purified by HPLC did not differ between starved and control rats. Furthermore, in the same sample values for Cmax per milligram protein of the starved rats were 90% of control values. These results suggest that the Cmax per TR molecule was not changed by starvation.

DISCUSSION

Starvation of rats for 72 hours decreases TR levels in the liver and kidney when measured by a T_3-binding assay.[4-7] However, it is not known whether the starvation-induced reduction of Cmax is due to the loss of TR proteins, since no change was found in rat hepatic TR mRNA levels during fasting.[16] In the present study, we measured TR protein

Table 2. Starvation-Induced Changes in Cmax and TR Protein Levels in Rat Liver

Group	Cmax (pg T_3)		TR Protein Level			
			Per mg Protein		Per mg DNA	
	Per mg Protein	Per mg DNA	47 kd	55 kd	47 kd	55 kd
Control (n = 5)	239.9 ± 38.4	158.1 ± 23.3	1.00 ± 0.05	1.00 ± 0.06	1.00 ± 0.06	1.00 ± 0.10
Starved (n = 5)	267.9 ± 43.6	114.6 ± 17.6*	0.99 ± 0.06	1.02 ± 0.21	0.64 ± 0.04†	0.66 ± 0.14†
	(111%)	(72.5%)				

NOTE. TR protein levels in starved rats are expressed relative to those in controls. Cmax for T_3 was measured by Scatchard plot analysis using the same aliquots used for Western blotting. Values are the mean ± SD.

*$P < .05$, †$P < .01$: *v* control.

Table 3. Starvation-Induced Changes in Cmax and TR Protein Levels in Rat Liver TR Fraction Purified by HPLC

Group	Cmax (pg T_3/mg protein)	TR Protein Level (per mg protein)	
		47 kd	55 kd
Control (n = 5)	681.7 ± 148.1	1.00 ± 0.16	1.00 ± 0.17
Starved (n = 6)	613.5 ± 125.7 (90.0%)	0.91 ± 0.14	0.85 ± 0.16

NOTE. Values for TR protein levels in starved rats are expressed relative to those in controls. Values are the mean ± SD.

content in rat liver by Western blotting, and found that the previously identified 47- and 55-kd TR proteins[18] were reduced by starvation. The decrease in TR protein levels expressed per milligram DNA correlated well with the decrease in Cmax per milligram DNA. Starvation-induced changes in TR protein and Cmax levels, as analyzed per milligram nuclear protein, were also well correlated in both the partially purified TR fraction and the crude nuclear extract. These results suggest that the Cmax per TR molecule was not altered by starvation.

The mechanism by which starvation reduced the levels of TR protein is unclear. Although starvation reduces hepatic poly(A)$^+$ RNA, ribosomal RNA, and polysomal complexes,[23,24] the level of TRβ1 mRNA, considered the predominant TR mRNA in rat liver,[15] does not change.[16] Starvation also changes plasma concentrations of glucagon and corticosterone,[25] both of which are known to affect T_3-binding activity.[26,27] Both proteolytic enzyme activity[28] and hepatic protein turnover[29,30] are enhanced by starvation, which may induce hepatic protein loss, and TR proteins are considered labile hepatic proteins.[31]

Dissociations between TR mRNA and Cmax have been reported. For example, Strait et al[15] demonstrated that in rat liver, Cmax is disproportionately higher than the TRα1 + β1 mRNA level, whereas the brain had high levels of TRα1 + β1 mRNA despite having a relatively low Cmax. Such a dissociation was also found during development in rat liver and brain[15] and in chick erythroid cells.[17] Thyroid hormone increases Cmax in both rat liver and kidney,[7] but not TR mRNA.[13,15,32] In our previous study, we showed that the Cmax per TR molecule is constant in rat liver and brain.[18] During starvation, Cmax is well correlated with TR protein level but not with TR mRNA level; therefore, the Cmax per TR molecule does not change.

The mechanism(s) by which TR protein levels dissociate from TR mRNA levels is not known at present. However, since the decrease in TR protein is not different from the decrease in total nuclear protein, the translational efficiency of TR mRNAs and/or the stability of the TR protein appear to be important. Determining whether the mechanism for the reduction in TR protein is different from that responsible for the general reduction in nuclear protein requires further investigation.

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