Effect of Thyroid Hormone on Peroxisomal Flavin Enzymes in Rat Liver and Kidney

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Abstract

The Effect of thyroid hormone on peroxisomal enzyme activity was studied in thyroidectomized- and T₄-administered-thyroidectomized rats. In liver, the activities of isozyme A of L- α -hydroxyacid oxidase, D-amino acid oxidase, urate oxidase and catalase were decreased by thyroidectomy, and the diminished enzyme activities were restored by T₄ administration to rats. These modifications induced by thyroidectomy or by T₄ administration, however, were prominent only in immature animals (20-day-old rats). Although the changes in α -hydroxyacid oxidase and D-amino acid oxidase activities, induced by thyroidectomy or by T₄ administration, were also observed in 40-day-old rats, those in urate oxidase and catalase activities were not significant in 40-day-old rats. Acyl CoA oxidase activity was not affected by thyroidectomy or by T₄ administration in either 20- or 40-day-old rats. In the kidney, isozyme B of L- α -hydroxyacid oxidase activity was reduced by thyroidectomy and the diminished enzyme activity was restored by T₄ administration in both 20- and 40-day-old rats. D-Amino acid oxidase and catalase activities in kidney, however, were not significantly modified by thyroidectomy or by T₄ administration in either 20- or 40-day-old rats. The results suggest that thyroid hormone can modify the peroxisomal enzyme activity, which is prominent in immature animals.

It is well known that flavin nucleotide enzymes are essential components of a number of oxidases, many of which utilize O₂. Thyroid hormone is known to regulate the conversion of riboflavin into coenzymes flavin mononucleotide and flavin adenine dinucleotide (Rivlin and Langdon, 1979; Fazekas *et al.*, 1978). They reported that

thyroid hormone stimulates the synthesis of these coenzymes by increasing the activity of flavokinase. On the other hand, it is uncertain whether the enzymes which require these coenzymes are influenced by thyroid hormone. Peroxisome is one of the organelles which are enriched in flavin coenzyme requiring enzymes (Dixon and Webb, 1979). Various enzymes, involved in fatty acid activation and oxidation (Lazarow and DeDuve, 1976; Bronfman *et al.*, 1979) and intracellular transport of fatty acyl moieties

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(Markwell *et al.*, 1972), have been localized in peroxisomes. In this study, we aimed to examine whether thyroid hormone can modify the activity of several oxidases which are present in peroxisomes.

Materials and Methods

Animal preparation

In experiment 1, immature male Wistar rats, weighing 50–60 g (20-day-old), were divided into two groups. One of them (5 rats) was sham-operated, and the other (17 rats) was thyroidectomized. The sham-operated rats were injected with saline subcutaneously for 7 days. The thyroidectomized-rats were divided into two subgroups. One of them (8 rats) was injected with saline for 7 days. The other (9 rats) was daily injected with thyroxine (T_4) (dissolved in saline containing 10^{-8} M NaOH) at a dose of 20 μ g/animal subcutaneously for the same period. Twelve h after the last injection of T_4 or saline, the animals were killed and trunk blood was collected into heparinized plastic tubes. The liver and kidney were removed and kept at -20°C until use. In experiment 2, male Wistar rats, weighing 100–105 g (40-day-old), were divided into two groups. One of them (5 rats) was injected with T_4 (20 μ g/animal) for 7 days and the other (4 rats) was injected with saline as described. Twelve h after the last injection, the blood, liver, and kidney specimens were prepared as described.

Enzyme assay

Livers and kidneys were homogenized with 5 volumes of 50 mM potassium phosphate buffer (pH 7.5), and the homogenates were used as an enzyme source. L- α -Hydroxyacid oxidase activity was assayed by measuring the rate of O_2 consumption with an oxygraph (Bioxygraph, Kyusui-Kagaku Inst. Tokyo, Japan) as previously described (Yokota *et al.*, 1985). The reaction mixture contained 0.1 M potassium phosphate buffer (pH 8.0), the substrate, and the enzyme in a final volume of 1.2 ml. The concentrations of the substrates were 20 mM glycolate for assay of isozyme A and 20 mM DL- α -hydroxybutyrate for assay of isozyme B. One μ g of bovine liver catalase was added to correct the effect of endogenous catalase activity on O_2 consumption.

Acyl-CoA oxidase was measured as previously described (Hashimoto *et al.*, 1981). D-Amino acid oxidase, urate oxidase and catalase were assayed by the method of Leighton *et al.* (1968). Enzyme assay was carried out at 30°C . One unit of enzyme activity was defined as the amount that catalyzed the reaction resulting in the consumption of 1 μ mole of substrate per min.

Quantitative precipitation reaction of L- α -Hydroxyacid oxidase

Preparation and purification of isozyme A and B of L- α -hydroxyacid oxidase and of their antibodies were performed as previously described (Yokota *et al.*, 1985). Liver and kidney were homogenized with three volumes of 50 mM potassium phosphate (pH 7.5), and centrifuged at $15,000 \times g$ for 20 min. The supernatants were used for quantitative precipitin reaction. Various amounts of the extract were added to the fixed amount of antibody. After incubation at 20°C for 30 min. in 0.15 M NaCl containing 50 mM potassium phosphate (pH 7.5), the reaction mixture was centrifuged. An aliquot of the supernatant was used for enzyme assay. The precipitate was washed once with 0.15 M NaCl-50 mM potassium phosphate (pH 7.5) containing 2% (v/v) Triton X-100, and three times with the same buffer without Triton X-100. The washed precipitate was subjected to protein assay.

Other analytical procedures

3, 5, 3'-Triiodo-L-thyronine (T_3) and T_4 concentrations in serum were measured by radio-immunoassays using commercially available kits (Kaken Kagaku, Tokyo, Japan) as previously described (Hiramatsu *et al.*, 1983). The protein concentration was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. Statistical significance was assessed by using Student's *t*-test.

Materials

T_4 , glycolate, DL- α -hydroxybutyrate, catalase and D-alanine were purchased from Sigma Chemical Co., (St. Louis, MO USA).

Results

As is shown in Table 1, body weight and

Table 1. Effects of thyroidectomy or T₄ administration to thyroidectomized rats on body weight, liver and kidney weights, and on serum T₃ and T₄ concentrations.

Treatment of rats	Body weight	Liver weight	Kidney Weight	T ₄ (μg/100 ml)	T ₃ (ng/100 ml)
Exp. 1					
Control (Sham-operated) (5)	88 ± 13	3.9 ± 0.6	1.1 ± 0.3	5.5 ± 1.3	75 ± 4
Thyroidectomized + Saline (8)	60 ± 9 ^a	1.8 ± 0.3 ^a	0.6 ± 0.1 ^a	1.2 ± 0.9 ^a	49 ± 17 ^b
Thyroidectomized + T ₄ (20 μg/day) (9)	98 ± 8 ^b	3.1 ± 0.5 ^b	1.0 ± 0.1 ^b	16.2 ± 4.6 ^b	501 ± 66 ^b
Exp. 2					
Control (Sham-operated) (5)	182 ± 19	7.8 ± 1.0	1.4 ± 0.1	5.4 ± 0.2	88 ± 11
Thyroidectomized + Saline (4)	95 ± 14 ^a	3.6 ± 0.8 ^a	0.7 ± 0.1 ^a	0.3 ± 0.2 ^a	35 ± 8 ^a
Thyroidectomized + T ₄ (20 μg/day) (5)	114 ± 5 ^b	4.4 ± 0.6 ^b	1.1 ± 0.1 ^b	10.5 ± 3.4 ^b	199 ± 29 ^b

The initial body weight of rats was 54 ± 4 g in exp. 1 and 102 ± 3 g in Exp. 2. Each value indicates the mean ± S. D. Parenthesis indicates the number of determinations. Explanations: a = p < 0.01 (compared to control); b = < 0.01 (compared to thyroidectomized animals).

Table 2. Effects of thyroidectomy or T₄ administration to thyroidectomized rats on peroxisomal enzyme activities in liver.

Treatment of rats	L-α-hydroxy- acid oxidase A	D-amino acid oxidases	Acyl CoA oxidase	Urate oxidase	Catalase (× 10 ⁻³ units/g liver)
(units/g liver)					
Exp. 1					
Control (Sham-operated) (5)	1.2 ± 0.2	0.28 ± 0.08	0.44 ± 0.21	1.1 ± 0.5	102 ± 17
Thyroidectomized + Saline (8)	0.3 ± 0.1 ^a	0.41 ± 0.06 ^a	0.41 ± 0.14 ^c	0.5 ± 0.2 ^a	48 ± 22 ^a
Thyroidectomized + T ₄ (20 μg/day) (9)	0.9 ± 0.2 ^b	0.43 ± 0.14 ^b	0.54 ± 0.23 ^d	1.0 ± 0.1 ^b	98 ± 19 ^b
Exp. 2					
Control (Sham-operated) (5)	1.1 ± 0.1	0.47 ± 0.04	0.33 ± 0.03	1.4 ± 0.1	72 ± 4
Thyroidectomized + Saline (4)	0.4 ± 0.1 ^a	0.26 ± 0.07 ^a	0.26 ± 0.05 ^c	1.2 ± 0.3 ^c	52 ± 9 ^e
Thyroidectomized + T ₄ (20 μg/day) (5)	0.8 ± 0.1 ^b	0.76 ± 0.15 ^b	0.38 ± 0.07 ^f	1.5 ± 0.2 ^d	60 ± 6 ^d

Each value indicates the mean ± S. D.. Parenthesis indicates the number of determinations. Explanations: a = < 0.01 (compared to control); b = < 0.01 compared to thyroidectomized animals); c = > 0.5 (compared to control); d = > 0.5 (compared to thyroidectomized rats); e = < 0.05 (compared to control); f = < 0.05 (compared to thyroidectomized rats).

liver and kidney weights were significantly decreased by thyroidectomy. Administration of T_4 to the thyroidectomized-animals prevented the reduction of these weights.

The low levels of serum T_3 and T_4 in thyroidectomized-animals and high levels of them in T_4 -injected-thyroidectomized-rats were observed in experiments 1 and 2.

Table 3. Effects of thyroidectomy or T_4 administration to thyroidectomized rats on peroxisomal enzyme activities in kidney.

Treatment of rats		L- α -hydroxyacid Oxydase B	D-amino acid oxidase	Catalase
		(units/g kidney)		($\times 10^{-3}$ units/g kidney)
Exp. 1				
Control				
(Sham-operated)	(5)	1.2 $\pm$ 0.2	4.1 $\pm$ 0.3	31 $\pm$ 6
Thyroidectomized + Saline	(8)	0.5 $\pm$ 0.2 ^a	3.8 $\pm$ 0.5	25 $\pm$ 3 ^c
Thyroidectomized + T ₄ (20 μ g/day)	(9)	1.3 $\pm$ 0.2 ^b	4.1 $\pm$ 0.3 ^d	28 $\pm$ 4 ^d
Exp. 2				
Control				
(Sham-operated)	(5)	0.74 $\pm$ 0.03	4.4 $\pm$ 0.5	24 $\pm$ 2
Thyroidectomized + Saline	(4)	0.36 $\pm$ 0.09 ^a	3.5 $\pm$ 1.1 ^c	17 $\pm$ 4 ^c
Thyroidectomized + T ₄ (20 μ g/day)	(5)	0.67 $\pm$ 0.14 ^b	4.6 $\pm$ 0.7 ^d	20 $\pm$ 2 ^d

Each value indicates the mean $\pm$ SD.. Parenthesis indicates the number of determinations. Explanations: a = <0.01 (compared to control); b = <0.01 (compared to thyroidectomized rat); c = >0.5 (compared to control); d = >0.5 (compared to thyroidectomized rats).

Table 4. Immunotitration of L- α -hydroxyacid oxydase activity.

Source of Enzyme	L- α -hydroxyacid oxydase A			L- α -hydroxyacid oxydase B		
	Activity added	Activity in supernatant	Protein precipitated	Activity added	Activity in supernatant	Protein precipitated
	(munits)		($A_{280 \text{ nm}}$)	(munits)		($A_{280 \text{ nm}}$)
Control rats	10	0	0.052	10	0	0.081
(Enzymes were prepared from rats in Exp. 1)	40	10	0.088	20	0	0.103
	85	41	0.060	47	31	0.088
Thyroidectomized + Saline	10	0	0.054	10	0	0.082
(Enzymes were prepared from rats in Exp. 2)	40	12	0.092	20	0	0.097
	85	43	0.063	47	29	0.062
Thyroidectomized + T_4	10	0	0.052	10	0	0.087
(Enzymes were prepared from rats in Exp. 1)	40	10	0.092	20	0	0.098
	85	41	0.067	47	31	0.073

Isozyme A and B were prepared from liver and kidney respectively. The antibodies to isozyme A and B were 0.63 mg and 0.73 mg respectively. Procedures for immunotitration are described in Materials and Methods.

Table 2 summarizes the levels of peroxisomal enzyme activity in liver. In experiments 1 and 2, the activity of L- α -hydroxyacid oxidase (isozyme A) was decreased by thyroidectomy. The activity of D-amino acid oxidase was also decreased by thyroidectomy. The diminished activity in these enzymes was restored by T₄ administration to the thyroidectomized-rats. The activity of D-amino acid oxidase was dramatically increased by T₄ administration in comparison with the increase in L- α -hydroxyacid oxidase activity. Urate oxidase and catalase activities were also decreased by thyroidectomy and the diminished activities were restored by T₄ administration in experiment 1. The T₄-induced increase in urate oxidase or catalase activity, however, was not significant in experiment 2. In contrast to these enzymes, acyl CoA oxidase activity was not strongly influenced by thyroidectomy or by T₄ administration to the animals in both 20- and 40-day-old rats.

Table 3 summarizes the peroxisomal enzyme activities in kidney. The activity of L- α -hydroxyacid oxidase (isozyme B) was decreased by thyroidectomy. The diminished enzyme activity was restored by T₄ administration to the thyroidectomized-animals. D-Amino acid oxidase and catalase activities, on the other hand, were not significantly affected by thyroidectomy or by T₄ administration to the animals though these activities were decreased by thyroidectomy and increased by T₄ administration in experiments 1 and 2.

Two isozymes of L- α -hydroxyacid oxidase were titrated by the corresponding antibodies. Table 4 shows that the enzyme activities in each liver and kidney tissue of any group were equally inactivated by the corresponding antibody. The equivalence points of the tissue extracts were the same as those of the purified enzyme preparations (Yokota *et al.*, 1985). The immunoprecipitates were analyzed by electrophoresis on a polyacrylamide gel in the presence of

sodium dodecylsulfate. The size and the intensity of staining of the subunits of the enzymes were the same in all groups of animals.

Discussion

In this preliminary report, we have demonstrated that thyroid hormone can modify the several peroxisomal enzyme activities in the liver and kidney. In immature (20-day-old) rats, L- α -hydroxyacid oxidase, D-amino acid oxidase, urate oxidase and catalase activities were significantly decreased by thyroidectomy and increased by thyroid hormone administration, whereas the activity of acyl CoA oxidase was not significantly affected by these treatments in the liver, suggesting that the effect of the hormone is enzyme-specific in peroxisome. The modification induced by thyroid hormone, however, appears to be related to the age of the animals. The thyroid hormone-induced increases in L- α -hydroxyacid oxidase and D-amino acid oxidase activities in 40-day-old rats were similar to those observed in 20-day-old rats, whereas the urate oxidase and catalase responses to thyroid hormone administration were less in 40-day-old rats than those observed in 20-day-old rats. As previously reported, the degree of thyroid hormone action in mitochondria is less in aged animals (Ichikawa *et al.*, 1982). The age-related changes in the response of hepatic enzymes to T₃ administration is also reported (Schwartz *et al.*, 1979). Thus, it is possible that thyroid hormone-induced modification of peroxisomal enzyme activities is diminished in aged animals.

As shown in this study, although L- α -hydroxyacid oxidase activity was increased by thyroid hormone in both the liver and kidney, D-amino acid oxidase activity which was significantly increased by the hormone in the liver was not strongly affected by thyroidectomy or by T₄ administration in the kidney. The Tissue-specific modification

induced by thyroid hormone was also observed in the mitochondrial protein synthesis system as previously reported (Ichikawa *et al.*, 1985). Based on these observations, it appears that the thyroid hormone action on peroxisome is also tissue (at least liver and kidney)-specific.

Although we did not measure the concentrations of enzymes, several reports (Rivlin and Langdon, 1979; Fazekas *et al.*, 1978) suggested that the amount of flavin coenzymes is increased by thyroid hormone, suggesting that one of the possible mechanisms by which thyroid hormone increase the activities of peroxisomal enzymes is related to the changes in the concentration of cofactors which are required to stabilize newly synthesized apoenzymes. Immunoprecipitation studies, however, showed that the thyroid hormone increases the net amount of enzymes. The results suggested that not only coenzymes but also several flavin enzymes are newly synthesized by thyroid hormone stimulation. However, further studies, such as quantitative analysis of changes in enzyme activity or in net amount of enzymes, will be required to solve the precise mechanism of stimulation of synthesis of the enzymes.

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