Thyroid Hormone and Activities of Drug-Metabolizing Enzymes and Electron Transport Systems of Rat Liver Microsomes

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SUMMARY

Drug-metabolizing activities of liver microsomes and the activities of microsomal electron transport systems were investigated in male and female rats with altered thyroid states.

The administration of thyroxine decreased the N-demethylation of aminopyrine and hydroxylation of hexobarbital by liver microsomes in male rats. In contrast, the same treatment increased the metabolism of aminopyrine and hexobarbital in female rats. The hydroxylation of aniline and reduction of *p*-nitrobenzoic acid were increased in both male and female rats.

The administration of thyroxine increased the activity of microsomal NADPH oxidase, NADPH-cytochrome *c* reductase, and NADPH-neotetrazolium reductase in both male and female rats, but the magnitude of increase was much greater in female rats than in male rats. The content of P-450 was decreased in male rats, but this content was not significantly altered in female rats.

The metabolism of aminopyrine, hexobarbital, aniline, and *p*-nitrobenzoic acid and the activities of NADPH oxidase, NADPH-cytochrome *c* reductase, NADPH-neotetrazolium reductase, and NADH oxidase were decreased in the thyroidectomized male and female rats, but the activity of NADH-cytochrome *c* reductase and cytochrome b_5 and the P-450 content were not significantly altered.

The administration of triiodothyronine completely restored all values in the thyroidectomized female rats, but the activities of aminopyrine N-demethylase, hexobarbital hydroxylase, and NADPH-neotetrazolium reductase and P-450 content remained low in the male rats.

The stimulative effect of phenobarbital administration on the microsomal enzymes was observed in the thyroxine-treated rats and thyroidectomized rats as well as in normal rats.

INTRODUCTION

Liver microsomal enzymes, called drug-metabolizing enzymes, can oxidize various lipid-soluble compounds in the presence of NADPH and oxygen. In a previous paper, we reported that the metabolism of aminopyrine and hexobarbital by the liver microsomes was markedly decreased by the administration of thyroxine in male rats, but was not altered, or even was slightly increased, in female rats (1). On the other hand, it is well known that the administration of thyroxine markedly increases the activity of NADPH-cytochrome *c* reductase of liver microsomes (2).

The activities of microsomal drug-metabolizing enzymes in various states have usually been correlated with the activities of microsomal NADPH oxidase, NADPH-cytochrome *c* reductase, and NADPH-neotetrazolium reductase (3–7). Moreover, several investigators have re-

cently presented evidence concerning a possible role of P-450 in the oxidation of drugs in liver microsomes (5–10). In the present investigation, therefore, a possible role of thyroid hormone for the regulation of the activities of drug-metabolizing enzymes was investigated in both female and male rats in relation to the activities of electron transport systems and P-450 content of rat liver microsomes.[1]

METHODS

Female and male rats of the Wistar strain, weighing about 160 and 190 g, respectively, were used. Hyperthyroid rats were prepared by treatment with *l*-thyroxine sodium (1000 μg/kg, i.p.) for 10 days. Hypothyroid rats were prepared by thyroidectomy 10 days or 23 days before the experiments.

The magnitude of hyper- or hypothyroidism was evaluated by determining the activity of mitochondrial cytochrome *c*-linked α-glycerophosphate dehydrogenase. Phenobarbital sodium, *l*-thyroxine sodium, and *l*-triiodothyronine sodium were dissolved in distilled water and given intraperitoneally as indicated in the tables and figures.

Preparation of microsomes. The rats were decapitated and the livers were removed, chopped into small pieces, washed well, and homogenized with 3 volumes of 1.15% (isotonic) KCl solution in a Teflon-glass homogenizer. The homogenate was centrifuged at 9000 *g* for 20 min. The supernatant solution was then centrifuged at 105,000 *g* for 1 hr, and the microsomes were suspended in 1.15% KCl solution. The possibility of mitochondrial contamination was excluded by the lack of succinate-cytochrome *c* reductase activity, according to Imai *et al.* (11, 12).

Assays of drug-metabolizing enzymes. The incubation mixture consisted of 9000 *g* supernatant equivalent to 600 mg of liver, 20 μmoles of glucose 6-phosphate, 0.6 μmole of NADP, 50 μmoles of nicotinamide, 50 μmoles of $MgCl_2$, 1.4 ml of 0.2 M sodium phosphate buffer (pH 7.4), various substrates (hexobarbital, 4.0 μmoles; aminopyrine, 5.0 μmoles; aniline, 5.0 μmoles; and *p*-nitrobenzoic acid, 5.0 μmoles), and water to a final volume of 5.0 ml.

The mixtures were incubated for 30 min under air with the exception of *p*-nitrobenzoic acid, which was incubated under nitrogen. The hydroxylation of hexobarbital was determined by measuring the disappearance of the substrates according to the method of Cooper and Brodie (13). The N-demethylation of aminopyrine was determined by measuring the formation of 4-aminoantipyrine according to the method of La Du *et al.* (14). The hydroxylation of aniline was determined by measuring the formation of *p*-aminophenol according to the method described in a previous paper (1). The reduction of *p*-nitrobenzoic acid was determined by measuring the formation of *p*-aminobenzoic acid according to the method of Fouts and Brodie (15). The metabolic activities were expressed as millimicromoles of substrate metabolized per gram wet weight of liver per 30 min.

Assays of components of electron transport systems in liver microsomes. NADPH oxidase was assayed spectrophotometrically according to the method of Gillette *et al.* (16). NADPH-cytochrome *c* reductase and NADPH-neotetrazolium reductase were determined according to the methods of Williams and Kamin (17). NADH oxidase was assayed spectrophotometrically by a method similar to that used for NADPH oxidase. NADH-cytochrome *c* reductase was assayed spectrophotometrically by a method similar to that used for NADPH-cytochrome *c* reductase. The enzyme activities were expressed as millimicromoles or micromoles metabolized per gram wet weight of liver.

Estimations of P-450 and cytochrome b_5. The contents of P-450 and cytochrome b_5 were determined by measuring the difference spectrum of a microsomal preparation in a Hitachi spectrophotometer with cuvettes of 1 cm optical path. A 1.6 ml sample of microsomal preparation, equiva-

[1] Part of this work was presented at the annual meeting of the Biochemical Society of Japan [*Seikagaku*, 38, 556 (1966)].

lent to 400 mg of liver, and 1.2 ml of 0.1 M phosphate buffer (pH 7.4) were placed in cuvettes (reference cell); sodium dithionate was added to another cuvette (sample cell I); the contents of a third cuvette were reduced with dithionate and saturated with carbon monoxide by gassing for 30 sec (sample cell II). The P-450 content was determined by the difference spectrum between sample cell II and sample cell I at 450 mμ and 500 mμ and expressed as millimicromoles per gram wet weight of liver according to Omura and Sato (18). The cytochrome b_5 content was determined by the difference spectrum between sample cell I and the reference cell at 424 mμ and 408 mμ and expressed as millimicromoles per gram wet weight of liver according to Omura and Sato (18).

Assay of mitochondrial cytochrome c-linked α-glycerophosphate dehydrogenase and microsomal and mitochondrial protein. After separation of the mitochondrial fraction the activity of cytochrome *c*-linked α-glycerophosphate dehydrogenase was determined by the method of Lee and Lardy (19). Microsomal and mitochondrial proteins were assayed by the method of Lowry *et al.* (20).

RESULTS

Effect of Thyroxine Administration on the Activities of Microsomal Drug-Metabolizing Enzymes in Male and Female Rats

The treatment with thyroxine increased the heart weight and the activity of mitochondrial cytochrome *c*-linked α-glycerophosphate dehydrogenase, both indexes commonly used as criteria of hyperthyroidism (19). Moreover, it did not alter protein content of whole homogenate and it very slightly (3–5%) increased microsomal protein in both male and female rats. Control values of microsomal protein were 27.3 ± 0.4 mg and 26.9 ± 0.5 mg per gram wet weight of liver, respectively, for male and female rats. As shown in Table 1 the administration of thyroxine decreased the N-demethylation of aminopyrine and hydroxylation of hexobarbital in male rats, but it increased the hydroxylation of aniline and did not alter the nitroreduction of *p*-nitrobenzoic acid.

On the other hand, the administration of thyroxine increased the metabolism of aminopyrine, hexobarbital, aniline, and *p*-nitrobenzoic acid in female rats.

TABLE 1

Effect of thyroxine administration on the metabolism of aminopyrine, hexobarbital, aniline, and p-nitrobenzoic acid by liver microsomes in male and female rats

The rats were treated with thyroxine (1 mg/kg, i.p.) for 10 days. Results are expressed as the mean ± SE of the values in mμmole/gram wet weight of liver/30 min. The numerals in parentheses indicate the number of animals used.

Drug	Controls	Thyroxine treated	Difference (%)
	Males		
Aminopyrine	542 ± 29 (18)	189 ± 13 (18)	−65**
Hexobarbital	2951 ± 128 (24)	1462 ± 81 (24)	−50**
Aniline	568 ± 15 (24)	783 ± 19 (24)	+38**
p-Nitrobenzoic acid	1251 ± 33 (17)	1195 ± 48 (17)	−4
	Females		
Aminopyrine	109 ± 6 (17)	177 ± 10 (17)	+62**
Hexobarbital	878 ± 59 (17)	1245 ± 98 (17)	+42**
Aniline	386 ± 12 (23)	593 ± 21 (23)	+54**
p-Nitrobenzoic acid	854 ± 38 (17)	1058 ± 61 (17)	+24**

** $p < 0.01$.

Effect of Thyroxine Administration on the Activities of Microsomal Electron Transport Systems in Male and Female Rats

The administration of thyroxine to male rats increased the activities of NADPH oxidase, NADPH-cytochrome *c* reductase, and NADPH-neotetrazolium reductase (Table 2). In contrast, the content of P-450 was decreased by the thyroxine treatment. The activities of NADPH oxidase, NADPH-cytochrome *c* reductase, and NADPH-neotetrazolium reductase were increased even more in the female rats, but the P-450 value decreased only slightly and the change was not statistically significant.

It was worthwhile to note that the sex differences in the activities of NADPH oxidase, NADPH-cytochrome *c* reductase, and NADPH-neotetrazolium reductase observed in the present investigation were more striking than those observed in a previous investigation made on rats of the Sprague-Dawley strain (21).

In addition, the content of P-450 was significantly higher in the male rats than in the female rats. The treatment with thyroxine increased the activity of NADH oxidase in both male and female rats (Table 3). In contrast, the activity of NADH-cytochrome *c* reductase decreased and the content of cytochrome b_5 was not significantly altered.

TABLE 2

Effect of thyroxine administration on the activities of microsomal NADPH-linked electron transport systems in male and female rats

The rats were treated with thyroxine (1 mg/kg, i.p.) for 10 days. Results are expressed as the means ± SE of values (mμmole/g/wet weight liver/3 min or 10 min). The numerals in parentheses indicate the number of animals used.

System	Controls	Thyroxine-treated	Difference (%)
	Males		
NADPH oxidase (mμmole/g/3 min)	1687 ± 59 (24)	2028 ± 81 (24)	+20**
NADPH-Cyt *c* reductase (μmole/g/3 min)	12.9 ± 0.9 (24)	15.7 ± 1.2 (24)	+22**
NADPH-NT reductase (μmole/g/10 min)	31.7 ± 1.9 (24)	44.4 ± 2.8 (18)	+40**
P-450 (mμmole/g)	28.8 ± 1.8 (24)	14.5 ± 1.3 (18)	−50**
	Females		
NADPH oxdase (mμmole/g/3 min)	1137 ± 48 (23)	1688 ± 89 (23)	+48**
NADPH-Cyt c reductase (μmole/g/3 min)	8.93 ± 0.6 (24)	14.1 ± 1.2 (24)	+58**
NADPH-NT reductase (μmole/g/10 min)	14.8 ± 0.8 (18)	39.2 ± 2.9 (18)	+165**
P-450 (mμmole/g)	21.2 ± 1.3 (23)	19.0 ± 1.9 (17)	−11

** $p < 0.01$.

Effect of Phenobarbital on the Activities of Microsomal Drug-Metabolizing Enzymes and Electron Transport Systems in Thyroxine-Treated Male Rats

It is well known that the administration of phenobarbital and other drugs increases the activity of microsomal drug-metabolizing enzymes as well as the activity of microsomal NADPH-linked electron transport systems (3–8, 22). In the present

TABLE 3

Effect of thyroxine administration on the activity of microsomal NADH-linked electron transport systems in male and female rats

The rats were treated with thyroxine (1 mg/kg, i.p.) for 10 days. Results are expressed as the means ± SE f values (mμmole/g wet weight liver/3 min). The numerals in parentheses indicate the number of animals sed.

System	Controls	Thyroxine-treated	Difference (%)
	Males		
NADH oxidase (mμmole/g/3 min)	776 ± 35 (12)	2604 ± 70 (12)	+198**
NADH-Cyt *c* reductase (μmole/g/3 min)	182 ± 7 (18)	129 ± 10 (18)	−29**
Cyt b_5 (mμmole/g)	17.9 ± 1.1 (18)	20.9 ± 1.4 (18)	+17*
	Females		
NADH oxidase (mμmole/g/3 min)	735 ± 62 (6)	1909 ± 106 (6)	+173**
NADH-Cyt *c* reductase (μmole/g/3 min)	171 ± 9 (18)	80 ± 6 (18)	−53**
Cyt b_5 (mμmole/g)	15.7 ± 0.8 (18)	17.7 ± 1.1 (18)	+13

* $p < 0.05$.
** $p < 0.01$.

study, we investigated whether the effect of phenobarbital may be modified in the thyroxine-treated male rats. The administration of phenobarbital to normal male rats increased the content of microsomal protein only about 9%, whereas it increased the activity (per gram wet weight of liver) of aminopyrine N-demethylase, hexobarbital hydroxylase, aniline hydroxylase, and *p*-nitrobenzoic acid nitroreductase to a much greater extent, as shown in Fig. 1.

The administration of phenobarbital to the thyroxine-treated male rats increased

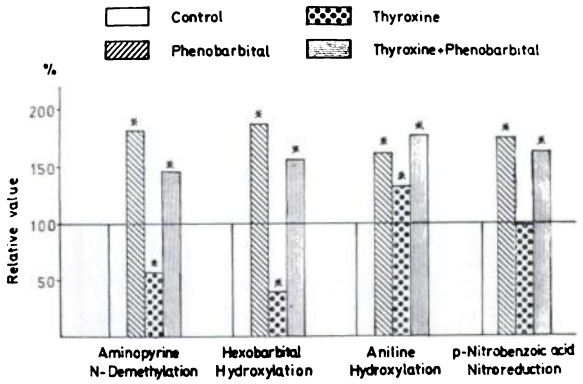

FIG. 1. *Effect of phenobarbital on the activities of microsomal drug-metabolizing enzymes in thyroxine-treated male rats*

Male rats were treated with 1 mg/kg (i.p.) of thyroxine for 10 days and (or) treated with 80 mg/kg (i.p.) of phenobarbital 72 hr and 48 hr before the sacrifice. The results are given as averages of values obtained from at least 12 rats and expressed as relative value (controls = 100). The asterisks in the figure indicate significant differences ($p < 0.05$) from controls.

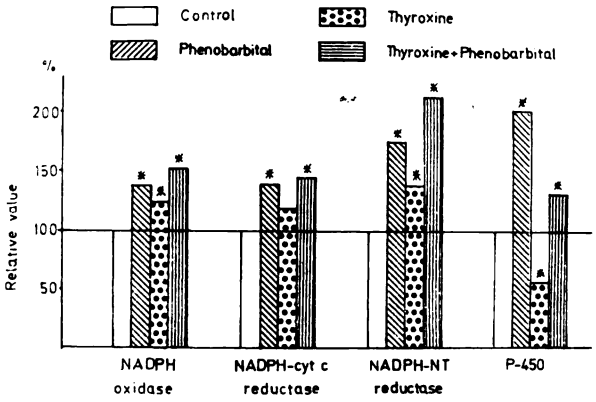

FIG. 2. *Effect of phenobarbital on the activities of microsomal NADPH-linked electron transport systems in thyroxine-treated male rats*

Experimental conditions as in legend to Fig. 1.

the content of microsomal protein only about 8%, whereas it increased the thyroxine-depressed activities of aminopyrine N-demethylase and hexobarbital hydroxylase to well above the control levels. Moreover, the activities of aniline hydroxylase and *p*-nitrobenzoic acid nitroreductase were increased in the thyroxine-treated rats as well as in normal rats. These results indicate that the induction of microsomal drug-metabolizing enzymes by phenobarbital occurred in the thyroxine-treated rats just as in normal rats.

The administration of phenobarbital to thyroxine-treated rats increased activities of NADPH oxidase, NADPH-cytochrome *c* reductase, NADPH-neotetrazolium reductase as well as in normal rats (Fig. 2). Moreover, the content of P-450 in the thyroxine-treated rats was increased over the control levels. The activity of NADH oxidase was decreased by the treatment with phenobarbital, and phenobarbital suppressed the thyroxine effect (Fig. 3). On the other hand, although the activity of NADH-cytochrome *c* reductase was decreased in the rats treated with phenobarbital or thyroxine alone, no further decrease was observed because of phenobarbital in the thyroxine-treated rats.

Effect of Thyroidectomy on the Activities of Microsomal Drug-Metabolizing Enzymes in Male and Female Rats

The activity of mitochondrial cytochrome *c*-linked α-glycerophosphate dehy-

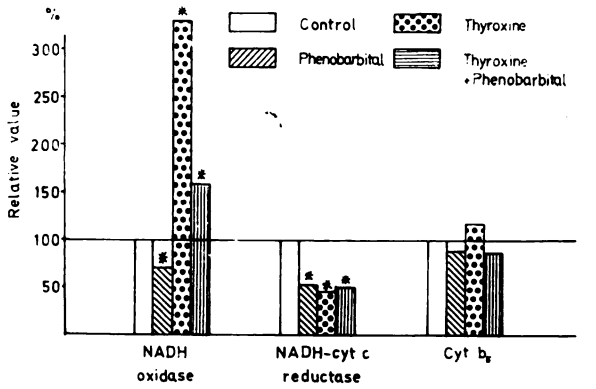

FIG. 3. *Effect of phenobarbital on the activity of microsomal NADH-linked electron transport systems in thyroxine-treated male rats*

Experimental conditions as in legend to Fig. 1.

TABLE 4

Effect of thyroidectomy and triiodothyronine (T_3) treatment on the activities of microsomal drug-metabolizing enzymes

Male and female rats were thyroidectomized 23 days before the sacrifice and half of the thyroidectomized rats were treated with triiodothyronine (100 μg/kg, i.p.) 48 hr and 24 hr before sacrifice. The results are given as averages of values obtained from at least 6 rats.

Drug	Group	Male rats	Difference	Female rats	Difference
Aminopyrine	Control	523 ± 48		135 ± 4	
(mμmole/g/30 min)	Thyroidectomy	204 ± 34	−60*	83 ± 5	−38*
	Thyroidectomy + T_3	199 ± 43	−62*	154 ± 8	+14
Hexobarbital	Control	2401 ± 139		673 ± 27	
(mμmole/g/30 min)	Thyroidectomy	1357 ± 194	−43*	540 ± 15	−20*
	Thyroidectomy + T_3	1734 ± 230	−28*	948 ± 103	+40*
Aniline	Control	514 ± 23		375 ± 5	
(mμmole/g/30 min)	Thyroidectomy	304 ± 17	−37*	273 ± 8	−27*
	Thyroidectomy + T_3	481 ± 18	−6	410 ± 9	+9*
p-Nitrobenzoic acid	Control	1538 ± 52		1176 ± 24	
(mμmole/g/30 min)	Thyroidectomy	1014 ± 104	−44*	868 ± 53	−26*
	Thyroidectomy + T_3	1491 ± 36	−3	1455 ± 61	+24*
Microsomal protein	Control	29.1 ± 0.2		26.5 ± 0.5	
(mg/g)	Thyroidectomy	25.7 ± 0.2	−12*	25.3 ± 0.4	−5
	Thyroidectomy + T_3	27.4 ± 0.4	−9	29.4 ± 1.2	+11*

* Significant difference ($p < 0.05$) from controls.

drogenase in thyroidectomized rats decreased to about 50% of control. As shown in Table 4, thyroidectomy decreased the activities of aminopyrine N-demethylase, hexobarbital hydroxylase, aniline hydroxylase and *p*-nitrobenzoic acid nitroreductase in male rats. Moreover, thyroidectomy decreased the activities of the four enzymes in female rats. The magnitudes of decrease in the metabolism of aniline and *p*-nitrobenzoic acid were almost the same in both male and female rats, but the decrease in metabolism of aminopyrine and of hexobarbital was more marked in male rats than in female rats.

Effect of Thyroidectomy on the Activities of Microsomal Electron Transport Systems in Male and Female Rats

The activities of microsomal NADPH oxidase, NADPH-cytochrome *c* reductase, and NADPH-neotetrazolium reductase were decreased in thyroidectomized male and female rats (Table 5). The activity of microsomal NADH oxidase was decreased in the thyroidectomized male and female rats, but the activity of NADH-cytochrome *c* reductase and the content of cytochrome b_5 were not significantly altered. In further experiments we observed similar results in the activities of microsomal enzymes and the content of P-450 and cytochrome b_5 in rats thyroidectomized 10 days previously.

Effect of Triiodothyronine or Thyroxine Administration on the Activities of Microsomal Drug-Metabolizing Enzymes in Thyroidectomized Male and Female Rats

It has been reported that the administration of triiodothyronine or thyroxine to thyroidectomized rats rapidly removed most signs of thyroidectomy within 2 or 3 days (23, 24). The administration of triiodothyronine to the thyroidectomized rats increased the activity of mitochondrial cytochrome *c*-linked α-glycerophosphate dehydrogenase over the control level and the activities of aniline hydroxylase and *p*-nitrobenzoic acid nitroreductase were completely restored; but the activities

TABLE 5
Effect of thyroidectomy and triiodothyronine (T_3) treatment on the activities of microsomal electron transport systems
Experimental conditions are as given in the legend for Table 4.

System	Group	Male rats	Difference	Female rats	Difference
NADPH oxidase	Control	1826 ± 100		1032 ± 106	
(mμmole/g/3 min)	Thyroidectomy	747 ± 124	−60*	676 ± 51	−36*
	Thyroidectomy + T_3	2056 ± 149	+12	1485 ± 142	+44
NADPH-cytochrome *c*	Control	11.1 ± 1.0		7.8 ± 0.6	
reductase	Thyroidectomy	4.6 ± 0.5	−59*	3.6 ± 0.2	−54*
(μmole/g/3 min)	Thyroidectomy + T_3	12.3 ± 2.3	+11	11.0 ± 0.6	+28*
NADPH-neotetrazolium	Control	36.4 ± 2.8		15.7 ± 0.8	
reductase	Thyroidectomy	9.2 ± 1.5	−75*	4.9 ± 0.4	−68*
(μmole/g/3 min)	Thyroidectomy + T_3	26.1 ± 1.9	−30*	20.0 ± 1.8	+20*
P-450 (mμmole/g)	Control	27.9 ± 1.0		22.4 ± 1.8	
	Thyroidectomy	24.1 ± 2.4	−14	25.3 ± 1.2	+13
	Thyroidectomy + T_3	18.0 ± 1.9	−35*	20.8 ± 1.2	−7
NADH oxidase	Control	723 ± 61		745 ± 79	
(mμmole/g/3 min)	Thyroidectomy	474 ± 67	−40*	286 ± 45	−61*
	Thyroidectomy + T_3	1486 ± 147	+105*	1298 ± 93	+73*
NADH-cytochrome *c*	Control	162 ± 23		157 ± 8	
reductase	Thyroidectomy	159 ± 7	−1	185 ± 15	+18
(μmole/g/3 min)	Thyroidectomy + T_3	138 ± 17	−15	162 ± 9	+3
Cytochrome b_5	Control	17.6 ± 0.5		19.8 ± 1.2	
(mμmole/g)	Thyroidectomy	17.9 ± 0.2	+2	16.8 ± 1.0	−15
	Thyroidectomy + T_3	16.6 ± 0.7	−6	17.4 ± 0.5	−12

* Significant difference ($p < 0.05$) from controls.

of aminopyrine N-demethylase and hexobarbital hydroxylase were not clearly restored (Table 4). The administration of thyroxine (300 μg/kg, 24 hr and 48 hr before the sacrifice) produced similar results. On the other hand, the administration of triiodothyronine to the thyroidectomized female rats stimulated the metabolism of aminopyrine, hexobarbital, aniline, and *p*-nitrobenzoic acid, as shown in Table 4.

Effect of Triiodothyronine or Thyroxine Administration on the Activities of Microsomal Electron Transport Systems in Thyroidectomized Male and Female Rats

The activities of NADPH oxidase, NADPH-cytochrome *c* reductase, and NADPH-neotetrazolium reductase in thyroidectomized female and male rats were increased by the administration of triiodothyronine (Table 5). In contrast, the content of P-450 in the thyroidectomized male rats was decreased by the treatment with triiodothyronine. The activity of NADH oxidase was increased, but the activity of NADH-cytochrome *c* reductase and the content of cytochrome b_5 were not altered in male or female rats by the treatment with triiodothyronine. The administration of thyroxine to the thyroidectomized rats produced similar results.

Effect of Phenobarbital on the Activities of Microsomal Drug-Metabolizing Enzymes and Electron Transport System in Thyroidectomized Female Rats

Amino acid incorporation by liver microsomes isolated from thyroidectomized rats is lower than that from control rats (23, 24). On the other hand, the increase in the activities of microsomal drug-metabolizing enzymes by phenobarbital is related to the increase in the incorporation of amino

acids into liver microsomal protein and microsomal enzymes (25–27). It was thus of interest to determine whether the phenobarbital-induced increase in the activities of microsomal drug-metabolizing enzymes and NADPH-dependent electron transport systems is modified or not in the thyroidectomized rats. As shown in Table 6 the percentage of increase in all the activities was higher in thyroidectomized rats than in normal rats. In contrast, the increase in P-450 content was smaller in the thyroidectomized rats.

DISCUSSION

The administration of thyroxine increases the rate of oxygen consumption and the activities of many enzymes in mitochondria (18, 24). Moreover, activities of microsomal enzymes such as NADPH-cytochrome *c* reductase and glucose 6-phosphatase are increased (2, 23). In contrast, the administration of thyroxine decreases the activities of aminopyrine N-demethylase and hexobarbital hydroxylase in male rats, but the activity of aniline hydroxylase is increased (1, 28). Moreover, the administration of thyroxine to female rats increases the activities of all three enzymes (1). On the other hand, it has been well established that the activities of microsomal drug-metabolizing enzymes are closely related to microsomal NADPH-linked electron transport systems (3–10, 16).

The present investigation presents the following evidence for the relationship between NADPH-linked electron transport systems and microsomal drug-metabolizing enzyme systems in relation to thyroid hormone.

The effects of thyroxine treatment. From the results given in Tables 1–3 it is evident that the sex difference in the alteration of the microsomal drug-metabolizing enzymes and electron transport systems by the

TABLE 6

Effect of phenobarbital on the activities of microsomal drug-metabolizing enzymes and electron transport system in thyroidectomized female rats

Female rats were thyroidectomized 23 days before sacrifice; half of them were treated with phenobarbital (60 mg/kg, i.p.) 72 hr and 48 hr before sacrifice. The results are given as averages of values obtained from at least 6 rats.

Drug or system	Thyroidectomy	Phenobarbital treatment		Percentage of increase
		−	+	
Aminopyrine (mμmole/g/30 min)	−	129 ± 8	632 ± 41	390*
	+	83 ± 5	587 ± 21	608*
Hexobarbital (mμmole/g/30 min)	−	745 ± 39	3151 ± 145	323*
	+	540 ± 15	3146 ± 17	484*
Aniline (mμmole/g/30 min)	−	392 ± 21	701 ± 39	79*
	+	273 ± 8	582 ± 46	114*
p-Nitrobenzoic acid	−	1190 ± 55	2225 ± 131	87*
(mμmole/g/30 min)	+	868 ± 53	1945 ± 163	124*
NADPH oxidase	−	985 ± 40	1517 ± 92	54*
(mμmole/g/3 min)	+	676 ± 51	1247 ± 114	86*
NADPH cytochrome *c* reductase	−	8.1 ± 0.4	15.0 ± 1.1	85*
(μmole/g/3 min)	+	3.6 ± 0.2	11.0 ± 0.8	209*
NADPH-neotetrazolium reductase	−	16.2 ± 0.8	31.3 ± 1.9	92*
(μmole/g/10 min)	+	4.9 ± 0.4	21.5 ± 2.5	330*
P-450 (mμmole/g)	−	21.7 ± 1.8	43.1 ± 8.5	99*
	+	25.3 ± 1.1	41.2 ± 4.4	63*
Microsomal protein (mg/g)	−	27.1 ± 0.5	30.3 ± 0.8	12*
	+	25.3 ± 0.4	30.3 ± 1.4	19*

* Significant difference ($p < 0.05$) from nontreated rats.

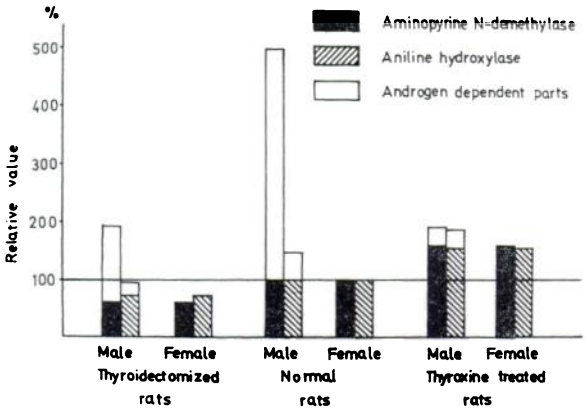

FIG. 4. *Schematic illustration of androgen-dependent and androgen-independent activities of drug-metabolizing enzymes in relation to altered thyroid function*

White bars indicate androgen-dependent parts of aminopyrine N-demethylase and aniline hydroxylase. Androgen-dependent parts were estimated by subtracting the value in female activities from that in male activities. The bars in the figure represent relative values (normal female = 100).

thyroxine treatment is related to the sex difference in their control values. For example, the effect of thyroxine on the enzyme activities which show a clear sex difference (e.g., aminopyrine N-demethylase and hexobarbital hydroxylase) was very different in male and female rats, whereas the effect of thyroxine on activities showing only a small sex difference (e.g., aniline hydroxylase and NADPH-cytochrome *c* reductase) was only slightly different. Moreover, the effect of thyroxine on the enzyme activities that show no sex difference (e.g., NADH oxidase and NADH-cytochrome *c* reductase) was not different in male and female rats.

It is thus possible to advance the hypothesis that the activities of aminopyrine N-demethylase and hexobarbital hydroxylase in male rats consist of two parts, one part representing basal activity and the other androgen-stimulated activity. As indicated in the schema of Fig. 4, the greater part of the activity of aminopyrine N-demethylase in male rats was androgen-stimulated whereas the androgen-stimulated activity formed only a small part of the aniline hydroxylase. The basal (androgen-independent) activities of aminopyrine N-demethylase and aniline hydroxylase were increased by the thyroxine treatment; in contrast, the androgen-dependent activities of these enzymes were decreased. The activities of aminopyrine N-demethylase in female rats and aniline hydroxylase in male rats, therefore, were increased by the thyroxine treatment; but in contrast, the activity of the androgen-stimulated aminopyrine N-demethylase in male rats was greatly decreased. Since the activity of hexobarbital hydroxylase shows similar sex differences to aminopyrine N-demethylase, the same mechanism may be involved. Sex differences in the effects of thyroxine similar to those seen with aniline hydroxylase were observed in the activities of NADPH oxidase and NADPH-cytochrome *c* reductase, so these may be interpreted similarly. Moreover, the clear sex difference in the effect of thyroxine observed in the activity of NADPH-neotetrazolium reductase may be interpreted in the same way. The presence of a sex difference and higher reactivity to thyroxine in the activity of NADPH-neotetrazolium reductase indicated a possibility that the reduction of cytochrome *c* and neotetrazolium by NADPH in liver microsomes may occur in a different manner, a possibility now under investigation in our laboratory.

The interference by thyroxine with the effect of androgen in stimulating the liver microsomal enzymes is probably not re-

lated to an increased breakdown of testosterone or a depression of testosterone production in the thyroxine-treated rats (29), since similar effects of thyroxine could be produced in castrated rats treated with high doses of androgen or anabolic hormone[2] (1). On the other hand, a threefold increase in the content of P-450 in thyroxine-treated rats was reported by Anes and da Silva (30). The reason for the discrepancy between their results and the present work is not known. However recent observations of Wada, made on thyroid-fed male rats, agree with the present results (31).

The effect of thyroidectomy. The activities of drug-metabolizing enzymes and NADPH-linked electron transport systems are significantly decreased in the thyroidectomized male and female rats, but the content of P-450 was not altered. The activities of all these enzymes were restored in female rats by the administration of triiodothyronine, but the activities of androgen-dependent enzymes in male rats were not restored to control levels. It is likely that the activities of both androgen-dependent and -independent parts were decreased in the thyroidectomized rats, but the magnitudes of the decrease were probably much greater in the androgen-dependent parts (Fig. 4).

It is probable that a suitable dose schedule of triiodothyronine will be needed for complete recovery of the androgen-dependent activities, such as aminopyrine N-demethylase, hexobarbital hydroxylase, and NADPH-neotetrazolium reductase.

On the other hand, Gillette (32) observed no significant alteration in the activities of microsomal drug-metabolizing enzymes in thyroidectomized rats, and Orrenius *et al.* (6) more recently observed no significant change in the activity of aminopyrine N-demethylase in the thyroidectomized male rats. The discrepancy between these results and the present investigation is not understood. In accord with the present investigation, Prang *et al.* recently reported a significant decrease in the rate of *in vivo* metabolism of pentobarbital and increased duration of narcosis in thyroidectomized male rats (33). We have also observed a marked increase in the duration of pentobarbital narcosis and of carisoprodol paralysis in thyroidectomized male and female rats.[3]

The effects of phenobarbital treatment. The administration of phenobarbital increased the activities of the microsomal NADPH-linked enzymes and the content of P-450 in the thyroxine-treated or thyroidectomized rats, even if their activities were decreased by the administration of thyroxine or by thyroidectomy. The magnitude of the phenobarbital-induced increase in the activities of the microsomal NADPH-linked enzymes was much greater in thyroidectomized rats than in normal rats. The absolute activities of the microsomal NADPH-linked enzymes were almost the same in thyroidectomized and normal rats treated with phenobarbital. Thus the enhanced effect of phenobarbital in thyroidectomized rats may be related to the low initial values.

It is clear from these results that there is a close relationship between the alterations in activities of drug-metabolizing enzymes and the NADPH-linked electron transport system in thyroxine-treated rats and thyroidectomized rats, provided the androgen-dependent component is taken into consideration (Fig. 4). However, the content of P-450 was not related to the activities of the other NADPH-linked components in thyroxine-treated rats and thyroidectomized rats. In the thyroxine-treated male rats the content of P-450 was apparently related to the activities of androgen-dependent drug-metabolizing enzymes. These results possibly indicate that the content of P-450 was rate limiting for the activities of androgen-dependent drug-metabolizing enzymes in thyroxine-treated rats. However, P-450 itself is not an enzyme, but plays a role in oxygen activation. Thus it is possible that there is no correlation between the activities of microsomal NADPH-linked electron transport

[2] R. Kato, unpublished observation.

[3] R. Kato, unpublished observation.

systems and the content of P-450 under the altered thyroid functions. Recently, many investigators have studied the alterations in the content of P-450 and have shown a relationship between activities of NADPH-linked electron transport systems and drug-metabolizing enzymes and the content of P-450 under some conditions. For example, the activities of the electron transport systems and drug-metabolizing enzymes and the content of P-450 were all increased by phenobarbital treatment (6–8) and starvation (6, 31, 34) and decreased by low protein diet (6, 33), refeeding (5) and in tumor-bearing (35) and old rats (36). However, the present investigation shows the necessity for determination of the activity of microsomal NADPH-P-450 reductase (37). A more clear relationship among the activities of NADPH-linked electron transport systems, the content of P-450, and activities of drug-metabolizing enzymes in normal rats as well as in rats with altered thyroid function may be obtained from such experiments.

REFERENCES

1. R. Kato and J. R. Gillette, *J. Pharmacol. Exptl. Therap.* **150,** 285 (1965).
2. A. H. Phillips and R. G. Lanfdon, *Biochim. Biophys. Acta* **19,** 380 (1956).
3. A. H. Conney and J. J. Burns, *Advan. Pharmacol.* **2,** 36 (1962).
4. R. Kato and J. R. Gillette, *Federation Proc.* **23,** 538 (1964).
5. R. Kato, *Japan. J. Pharmacol.* **17,** 181 (1967).
6. S. Orrenius, J. L. E. Ericsson and L. Ernster, *J. Cell Biol.* **25,** 627 (1965).
7. R. Kato, *J. Biochem.* **59,** 574 (1966).
8. H. Remmer and H. J. Merker, *Ann. N.Y. Acad. Sci.* **123,** 79 (1965).
9. T. Omura, R. Sato, D. Y. Cooper, O. Rosenthal and R. W. Estabrook, *Federation Proc.* **24,** 1181 (1965).
10. H. Remmer, J. Schenkman, R. W. Estabrook, H. Sasame, J. R. Gillette, S. Narasimhulu, D. Y. Cooper and O. Rosenthal, *Mol. Pharmacol.* **2,** 187 (1966).
11. Y. Imai, A. Ito and R. Sato, *J. Biochem.* **60,** 417 (1966).
12. E. Stolz, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. II, p. 740. Academic Press, New York, 1955.
13. J. R. Cooper and B. B. Brodie, *J. Pharmacol. Exptl. Therap.* **114,** 409 (1955).
14. B. N. La Du, L. Gaudette, N. Trousof and B. B. Brodie, *J. Biol. Chem.* **214,** 741 (1955).
15. J. R. Fouts and B. B. Brodie, *J. Pharmacol. Exptl. Therap.* **119,** 197 (1957).
16. J. R. Gillette, B. B. Brodie and B. N. La Du, *J. Pharmacol. Exptl. Therap.* **119,** 532 (1957).
17. C. H. Williams, Jr. and H. Kamin, *J. Biol. Chem.* **237,** 587 (1962).
18. T. Omura and R. Sato, *J. Biol. Chem.* **239,** 2370 (1964).
19. Y. Lee and H. A. Lardy, *J. Biol. Chem.* **240,** 1427 (1965).
20. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193,** 265 (1951).
21. R. Kato and J. R. Gillette, *J. Pharmacol. Exptl. Therap.* **150,** 279 (1965).
22. H. Remmer and H. J. Merker, *Science* **142,** 1657 (1963).
23. J. R. Tata, L. Ernster, O. Lindberg, E. Arrhenius, S. Pedersen and R. Hedman, *Biochem. J.* **86,** 408 (1963).
24. J. R. Tata, *in* "Actions of Hormones on Molecular Processes" (G. Litwack and D. Kritshevsky, eds.), p. 58. Wiley, New York, 1964.
25. R. Kato, L. A. Loeb and H. V. Gelboin, *Biochem. Pharmacol.* **14,** 1164 (1965).
26. R. Kato, W. R. Jondorf, L. A. Loeb, T. Ben and H. V. Gelboin, *Mol. Pharmacol.* **2,** 171 (1966).
27. H. Jick and H. Schuster, *J. Biol. Chem.* **241,** 5366 (1966).
28. A. H. Conney and L. Garren, *Biochem. Pharmacol.* **6,** 257 (1961).
29. J. S. McGuire, Jr. and G. M. Tomkins, *J. Biol. Chem.* **234,** 791 (1959).
30. R. I. Anes and A. da Silva, *Exptl. Cell Res.* **40,** 677 (1965).
31. F. Wada, *Seikagaku* **38,** 818 (1966).
32. J. R. Gillette, *Progr. Drug Res.* **6,** 13 (1963).
33. A. J. Prang, M. A. Lipton, R. B. Shearin and G. N. Love, *Biochem. Pharmacol.* **15,** 237 (1966).
34. R. Kato, *Biochem. Pharmacol.* **16,** 871 (1967).
35. R. Kato, A. Takanaka, A. Takahashi and K. Onoda, *Japan. J. Pharmacol.* in press.
36. R. Kato and A. Takanka, *Japan. J. Pharmacol.* in press.
37. T. Omura, E. Sanders, R. W. Estabrook, D. Y. Cooper and O. Rosenthal, *Arch. Biochem. Biophys.* **117,** 660 (1966).