SPECIES DIFFERENCES IN THE ALTERATION OF DRUG-METABOLIZING ACTIVITIES OF LIVER MICROSOMES BY THYROXINE TREATMENT

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The activities of drug-metabolizing enzymes of rat liver microsomes were altered by some unphysiological conditions (1-7). In previous papers we reported that the activities of hexobarbital hydroxylase and aminopyrine N-demethylase, which showed clear sex difference, were markedly decreased in the thyroxine treated male rats, whereas in female rats these activities were increased and the activity of aniline hydroxylase, which showed no clear sex difference, was increased in both male and female (3, 6). Similar results as observed in normal rats were obtained in male and female rats gonadectomized and treated with androgenic or anabolic hormones (3). These results, thus, indicate that the activities of hexobarbital hydroxylase and aminopyrine N-demethylase are dependent upon the anabolic action of male sex hormone and thyroxine probably decreases the androgen stimulated activity (3, 6).

On the other hand, it has been supposed that the androgen-dependent regulation mechanism of microsomal drug-metabolizing activity is likely present only in rats, but it is lack in other species of animals (8-10). Thus, it is of interest to investigate whether or not the sex difference in the effect of thyroxine on the drug-metabolizing enzymes is only observed in rats and the effect of thyroxine in other species of male and female is similar to that observed in female rats. The possibility of this assumption was investigated with both male and female rats, mice and rabbits and reported in the present paper. Moreover, since the activity of drug-metabolizing enzymes was connected with the activity of NADPH-linked electron transport system of liver microsomes, the correlation of both activities under the thyroxine treatment was also investigated (4-7, 11-15).

MATERIALS AND METHODS

Male and female rats of Wistar strain, weighing about 200 g and 160 g, respectively, and male and female rabbits, weighing about 2.2 kg and 2.0 kg, respectively, and male and female mice of *dd* strain, weighing about 22 g and 20 g, respectively, were used.

Preparation of microsomes: The animals were decapitated and the livers were removed, chopped into small pieces, washed well, and homogenized with 3 volumes of 1.15% (iso-

tonic) KCl solution in a Teflon-glass homogenizer. The homogenates were centrifuged at $9,000\times g$ for 20 minutes. The supernatant solutions were then centrifuged at $105,000\times g$ for one hour, and the microsomes were suspended in 1.15% KCl solution.

Assays of drug-metabolizing activities: The incubation mixtures consisted of $9,000\times g$ supernatant equivalent to 625 mg of liver, 20 μ moles of glucose-6-phosphate, 0.6 μ moles of NADP, 50 μ moles nicotinamide, 50 μ moles of $MgCl_2$, 1.4 ml of 0.2M sodium phosphate buffer (pH 7.4), various substrates (hexobarbital, 4.0 μ moles; aminopyrine, 5.0 μ moles; aniline, 5.0 μ moles; *p*-nitrobenzoic acid, 5.0 μ moles) and water to a final volume of 5.0 ml. The mixtures were incubated at 37°C for 30 minutes under air with exception of *p*-nitrobenzoic acid which was incubated in an atmosphere of nitrogen.

The hydroxylation of hexobarbital was determined by measuring the disappearance of the substrate according to the method of Cooper and Brodie (16). The N-demethylation of aminopyrine was determined by measuring the formation of 4-aminoantipyrine according to the method of La Du *et al.* (17). The hydroxylation of aniline was determined by measuring the formation of *p*-aminophenol according to the method described in a previous paper (2). The nitroreduction of *p*-nitrobenzoic acid was determined by the formation of *p*-aminobenzoic acid according to the method of Fouts and Brodie (18).

Assays of activities of electron transport systems in liver microsomes: NADPH oxidase was assayed spectrophotometrically according to the method of Gillette *et al.* (19). NADPH-cytochrome c reductase was determined according to the method of Williams and Kamin (20). NADPH-neotetrazolium reductase was determined according to the method described in a previous paper (15). NADH-cytochrome c reductase was determined according to the method similar to that of NADPH-cytochrome c reductase. The content of cytochrome P-450 and b_5 was determined as described in a previous paper (6).

Determination of microsomal and homogenate proteins: Liver microsomal and homogenate proteins were measured according to the method of Lowry *et al.* (21).

RESULTS

1. Effect of thyroxine administration on body weight, liver weight and heart weight in rats, mice and rabbits

Intraperitoneal administration of thyroxine (1.0 mg/kg, rats; 1.6 mg/kg, mice; 0.2 mg/kg, rabbits) markedly reduced body weight gain of all animals. The decrease in the body weight of rabbits was much greater than that of mice and rats. The percentage of body weight change by the thyroxine treatment in female mice and rats was +12.7% and +2.7%, respectively, while that of control female mice and rats was +15.7% and +10.8%, respectively.

On the other hand, the percentage of body weight change in female rabbits was -12.3%, while that of control female rabbits was +11.5%. The body weight of male rats was decreased more markedly than that of female rats, whereas no clear sex difference was observed in mice and rabbits. The liver weights of these animals were not significantly altered, thus the liver to body weight ratios were slightly increased. The treatment with

TABLE 1. Effect of thyroxine on heart weight in rats, mice and rabbits.

Species	Sex	Thyroxine treatment	Heart weight (g)	Differ. (%)	Heart weight / Body weight $\times 100$	Differ. (%)
Rats	Male	—	0.88 $\pm$ 0.03 (6)		0.29 $\pm$ 0.02 (6)	
	Male	—	1.31 $\pm$ 0.07 (6)	+49*	0.45 $\pm$ 0.02 (6)	+52*
	Female	—	0.69 $\pm$ 0.02 (6)		0.34 $\pm$ 0.02 (6)	
	Female	+	0.86 $\pm$ 0.03 (6)	+25*	0.43 $\pm$ 0.03 (6)	+26*
Mice	Male	—	0.071 $\pm$ 0.004 (12)		0.36 $\pm$ 0.02 (12)	
	Male	+	0.125 $\pm$ 0.005 (12)	+76*	0.68 $\pm$ 0.18 (12)	+89*
	Female	—	0.079 $\pm$ 0.003 (12)		0.40 $\pm$ 0.01 (12)	
	Female	+	0.117 $\pm$ 0.004 (12)	+49*	0.61 $\pm$ 0.02 (12)	+53*
Rabbits	Male	—	6.1 $\pm$ 0.3 (8)		0.23 $\pm$ 0.08 (8)	
	Male	+	6.6 $\pm$ 0.1 (8)	+ 8	0.32 $\pm$ 0.02 (8)	+39*
	Female	—	5.9 $\pm$ 0.2 (8)		0.25 $\pm$ 0.04 (8)	
	Female	+	6.8 $\pm$ 0.1 (8)	+15*	0.34 $\pm$ 0.01 (8)	+38*

Rats, mice and rabbits were intraperitoneally treated with thyroxine (1.0 mg/kg, 1.6 mg/kg and 0.2 mg/kg, respectively) for 10 days and the animals were sacrifice 24 hours after the last injection.

The results were expressed as averages $\pm$ S.E. The figures in parentheses indicate numbers of the determinations.

The stars in the table indicate the significant difference ($p < 0.05$) from controls.

TABLE 2. Effect of thyroxine on aminopyrine N-demethylation and hexobarbital hydroxylation by liver microsomes of rats, mice and rabbits.

Species	Sex	Thyroxine treatment	Aminopyrine N-demethylation (m μ mole/g/30 min)	Differ. (%)	Hexobarbital hydroxylation (m μ mole/g/30 min)	Differ. (%)
Rats	Male	—	542 $\pm$ 29 (18)		2951 $\pm$ 128 (24)	
	Male	+	189 $\pm$ 13 (18)	—65*	1462 $\pm$ 81 (24)	—50*
	Female	—	109 $\pm$ 6 (17)		878 $\pm$ 59 (17)	
	Female	+	177 $\pm$ 10 (17)	+62*	1245 $\pm$ 98 (17)	+42*
Mice	Male	—	304 $\pm$ 15 (17)		1253 $\pm$ 44 (17)	
	Male	+	141 $\pm$ 12 (17)	—54*	733 $\pm$ 56 (17)	—42*
	Female	—	247 $\pm$ 14 (16)		1213 $\pm$ 45 (16)	
	Female	+	195 $\pm$ 8 (17)	—21*	684 $\pm$ 70 (17)	—44*
Rabbits	Male	—	268 $\pm$ 18 (9)		1737 $\pm$ 143 (9)	
	Male	+	182 $\pm$ 17 (9)	—32*	1627 $\pm$ 72 (9)	— 6
	Female	—	268 $\pm$ 21 (9)		1580 $\pm$ 99 (9)	
	Female	+	153 $\pm$ 16 (9)	—43*	1576 $\pm$ 146 (9)	0

Rats, mice and rabbits were intraperitoneally with thyroxine (1.0 mg/kg, 1.6 mg/kg and 0.2 mg/kg, respectively) for 10 days and the animals were sacrifice 24 hours after the last injection.

The pooled liver from 2-3 mice were used for one determination. The results were expressed as averages $\pm$ S.E. The figures in parentheses indicate numbers of the determinations.

The stars in the table indicate significant difference ($p < 0.05$) from controls. The activity was expressed as m μ mole the substrate metabolized or metabolite formed by gram wet weight of liver.

thyroxine significantly increased the heart weight of rats, mice and rabbits. Thus, the ratios of heart to body weight were markedly increased in these animals (Table 1).

2. *Effect of thyroxine administration on homogenate and microsomal protein in liver of rats, mice and rabbits*

The administration of thyroxine did not result in any change in the liver protein content, but the content of microsomal protein showed a tendency of slight increase (less than 10%) or was not significantly altered in all animals used.

3. *Effect of thyroxine administration on the activities of drug-metabolizing enzymes of liver microsomes in rats, mice and rabbits*

The activity of aminopyrine N-demethylation was markedly decreased in male rats, but it was increased in female rats by the thyroxine treatment. On the other hand, the activity of aminopyrine N-demethylation in male and female mice and rabbits was decreased by the thyroxine treatment (Table 2).

The activity of hexobarbital hydroxylation was markedly decreased in male rats, but it was increased in female rats by the thyroxine. On the other hand, the activity of hexobarbital hydroxylation in male and female mice was decreased and the activity in rabbits was not significantly altered (Table 2). As reported in a previous paper, the sex differences in the effect of thyroxine on aminopyrine N-demethylation and hexobarbital hydroxylation are likely related to the evidence that the activities of these enzymes are androgen dependent and they are easily decreased in some unphysiological conditions (2-7).

It is of interest to note that there were no clear sex differences in the effect of thyroxine on the activities of aminopyrine N-demethylation and hexobarbital hydroxylation in mice and rabbits in accordance with the lack of clear sex differences in control activities. The activity of aniline hydroxylation was increased in male and female rats and rabbits by

TABLE 3. Effect of thyroxine on aniline hydroxylation and *p*-nitrobenzoic acid reduction by liver microsomes of rats, mice and rabbits.

Species	Sex	Thyroxine treatment	Aniline hydroxylation (mμmole/g/30 min)	Differ. (%)	<i>p</i> -Nitrobenzoic acid reduction (mμmole/g/30 min)	Differ. (%)
Rats	Male	—	568 ± 15 (24)		1251 ± 33 (17)	
	Male	+	783 ± 19 (24)	+ 38*	1195 ± 48 (17)	— 4
	Female	—	386 ± 12 (23)		854 ± 21 (17)	
	Female	+	593 ± 21 (23)	+ 54*	1058 ± 61 (17)	+ 24*
Mice	Male	—	1150 ± 37 (17)		1003 ± 38 (11)	
	Male	—	589 ± 31 (17)	— 49*	941 ± 39 (11)	— 6
	Female	—	945 ± 42 (16)		1036 ± 72 (11)	
	Female	+	594 ± 31 (17)	— 37*	953 ± 77 (11)	— 8
Rabbits	Male	—	462 ± 41 (9)		874 ± 53 (9)	
	Male	+	719 ± 43 (9)	+ 56*	1245 ± 69 (9)	+ 42*
	Female	—	461 ± 26 (9)		881 ± 45 (9)	
	Female	+	743 ± 44 (9)	+ 61*	1125 ± 78 (9)	+ 29*

See the legends for Table 2.

thyroxine, in contrast, it was decreased in male and female mice (Table 3).

The activity of *p*-nitrobenzoic acid nitroreduction was not altered in male rats, male and female mice, but it was slightly increased in female rats and male and female rabbits.

4. *Effect of thyroxine administration on the activities of electron transport systems of liver microsomes in rats, mice and rabbits*

The activity of NADPH oxidase was slightly increased in rats and it was markedly increased in rabbits, on the other hand, in mice the activity was not significantly altered

TABLE 4. Effect of the thyroxine on the activities of NADPH oxidase and NADPH-neotetrazolium (NT) reductase in liver microsomes of rats, mice and rabbits.

Species	Sex	Thyroxine treatment	NADPH oxidase (mμmole/g/3 min)	Differ. (%)	NADPH-NT reductase (μmole/g/10 min)	Differ. (%)
Rats	Male	—	1687 ± 59 (24)		31.7 ± 1.9 (24)	
	Male	+	2028 ± 81 (24)	+ 20*	44.4 ± 2.8 (18)	+ 40*
	Female	—	1137 ± 48 (23)		14.8 ± 0.8 (18)	
	Female	+	1688 ± 89 (23)	+ 40*	39.2 ± 2.9 (18)	+ 165*
Mice	Male	—	1220 ± 66 (11)		14.4 ± 0.8 (11)	
	Male	+	1453 ± 123 (11)	+ 19	14.3 ± 1.0 (11)	— 1
	Female	—	1346 ± 82 (11)		13.4 ± 0.9 (11)	
	Female	+	1430 ± 105 (11)	+ 6	13.0 ± 0.8 (11)	— 3
Rabbits	Male	—	437 ± 35 (8)		9.4 ± 1.2 (8)	
	Male	+	880 ± 111 (8)	+ 101*	24.7 ± 2.5 (8)	+ 162*
	Female	—	486 ± 51 (8)		10.0 ± 0.6 (8)	
	Female	+	1045 ± 242 (8)	+ 115*	23.9 ± 1.6 (8)	+ 139*

See the legends for Table 2.

The activity was expressed as mμmole NADPH oxidized or μmole neotetrazolium reduced by gram wet weight of liver.

TABLE 5. Effect of thyroxine on the activities of NADPH-cyt c reductase and NADH-cyt c reductase in liver microsomes of rats, mice and rabbits.

Species	Sex	Thyroxine treatment	NADPH-cyt c reductase (μmole/g/3 min)	Differ. (%)	NADH-cyt c reductase (μmole/g/3 min)	Differ. (%)
Rats	Male	—	12.9 ± 0.9 (24)		182 ± 7 (18)	
	Male	+	15.7 ± 1.2 (24)	+ 22*	129 ± 10 (18)	— 29*
	Female	—	8.9 ± 0.6 (24)		171 ± 9 (18)	
	Female	+	14.1 ± 1.2 (24)	+ 58*	80 ± 6 (18)	— 53*
Mice	Male	—	6.73 ± 0.52 (15)		84 ± 3 (7)	
	Male	+	7.95 ± 0.85 (15)	+ 16	74 ± 3 (7)	— 14*
	Female	—	7.28 ± 0.67 (15)		98 ± 8 (7)	
	Female	+	7.65 ± 0.64 (15)	+ 5	76 ± 4 (7)	— 24*
Rabbits	Male	—	6.58 ± 0.57 (8)		70 ± 7 (4)	
	Male	+	12.53 ± 0.85 (8)	+ 90*	39 ± 3 (4)	— 44*
	Female	—	6.76 ± 0.48 (8)		68 ± 4 (4)	
	Female	+	11.72 ± 0.73 (8)	+ 73*	40 ± 4 (4)	— 42*

See the legends for Table 2.

The activity was expressed as μmole cyt c reduced by gram wet weight of liver.

TABLE 6. Effect of thyroxine on the content of cyt P-450 and cyt b_5 in liver microsomes of rats, mice and rabbits.

Species	Sex	Thyroxine treatment	Cyt P-450 (m μ mole/g)	Differ. (%)	Cyt b_5 (m μ mole/g)	Differ. (%)
Rats	Male	—	27.9 $\pm$ 15 (18)		18.9 $\pm$ 1.2 (18)	
	Male	+	17.3 $\pm$ 17 (18)	—48*	19.1 $\pm$ 1.5 (18)	+1
	Female	—	21.2 $\pm$ 13 (23)		17.5 $\pm$ 0.9 (18)	
	Female	+	19.0 $\pm$ 19 (17)	—11	17.7 $\pm$ 1.2 (18)	+1
Mice	Male	—	30.7 $\pm$ 1.4 (11)		10.2 $\pm$ 0.5 (8)	
	Male	+	19.8 $\pm$ 1.0 (11)	—36*	9.9 $\pm$ 0.9 (8)	—3
	Female	—	26.6 $\pm$ 1.2 (11)		10.2 $\pm$ 0.9 (8)	
	Female	+	20.2 $\pm$ 1.0 (11)	—24*	9.6 $\pm$ 0.8 (8)	—6
Rabbits	Male	—	37.5 $\pm$ 1.9 (8)		18.8 $\pm$ 1.2 (8)	
	Male	+	30.1 $\pm$ 1.8 (7)	—21*	17.1 $\pm$ 0.9 (8)	—9
	Female	—	37.9 $\pm$ 3.5 (8)		19.1 $\pm$ 1.7 (8)	
	Female	—	28.1 $\pm$ 2.1 (8)	—26*	20.8 $\pm$ 2.1 (8)	+9

See the legends for Table 2.

The content was expressed as m μ mole cyt in gram wet weight of liver.

FIG. 1. Effect of various doses of thyroxine on the ratio of heart weight to body weight in rats, mice and rabbits.

Rats, mice and rabbits were treated with three different doses of thyroxine for 10 days and the animals were sacrificed 24 hours after the last injection. Rats (female) : low dose, 1.0 mg/kg ; middle dose, 2.0 mg/kg ; high dose, 4.0 mg/kg

Rats (male) : low dose, 0.5 mg/kg ; middle dose, 1.0 mg/kg ; high dose, 2.0 mg/kg

Mice : low dose, 0.8 mg/kg ; middle dose, 1.6 mg/kg ; high dose, 3.2 mg/kg

Rabbits : low dose, 0.05 mg/kg ; middle dose, 0.2 mg/kg

The results were expressed as variations to control values obtained from 5-24 animals.

The stars in the figure indicate the significance difference ($p < 0.05$) from controls.

(Table 4). The activity of NADPH-neotetrazolium reductase was increased in male and female rats, but the percentage was much greater in female rats. Moreover, the activity was greatly increased in male and female rabbits, but it was not altered in mice (Table 4.) The activity of NADPH-cytochrome c reductase was increased in male and female rats and rabbits, on the other hand, in mice the activity was not significantly altered (Table 5). On the other hand, the activity of NADH-cytochrome c reductase was decreased in the all animals investigated (Table 5). Cytochrome P-450 is an oxygen-activating component of liver microsomes for drug oxidation (15, 16, 22, 23), the content of cytochrome P-450 was decreased in male rats, but not in female rats.

Moreover, the content was decreased in male and female mice and rabbits (Table 6). On the other hand, the content of cytochrome b_5 , which is not involved in the oxidation of drugs, was not significantly altered in all the animals investigated (Table 6).

5. *Effect of the treatment with various doses of thyroxine on the activities of drug-metabolizing enzymes of liver microsomes in rats, mice and rabbits*

Since susceptibilities of rats, mice and rabbits against to thyroxine were markedly differed, the possibility occurred that the species differences observed in the present investigation may be due to only the matter of dosage of thyroxine.

Thus, the effect of thyroxine was investigated by changing the dose of the administ-

FIG. 2. Effect of various doses of thyroxine on aminopyrine N-demethylation by liver microsomes of rats, mice and rabbits.

Rats, mice and rabbits were treated with three different doses of thyroxine for 10 days and the animals were sacrificed 24 hours after the last injection. Rats (female) : low dose, 1.0 mg/kg ; middle dose, 2.0 mg/kg ; high dose, 4.0 mg/kg

Rats (male) : low dose, 0.5 mg/kg ; middle dose, 1.0 mg/kg ; high dose, 2.0 mg/kg

Mice : low dose, 0.8 mg/kg ; middle dose, 1.6 mg/kg ; high dose, 3.2 mg/kg

Rabbits : low dose, 0.05 mg/kg ; middle dose, 0.2 mg/kg

The results were expressed as relative values (control=100) obtained from 4-8 determinations.

The stars in the table indicate significance difference ($p < 0.05$) from controls.

ration. The percentage in the decrease of body weight by thyroxine was the minimum in mice and there was not clear difference among the three different dose. The rabbits was the most sensitive to thyroxine and with the high dose (0.5 mg/kg) 20–40 per cent of rabbits were died. The ratio of heart weight to body weight of various animals was given in Fig. 1. The ratio was markedly increased in the mice even though in the low dose group.

N-Demethylation of aminopyrine was decreased in male rats, male and female mice, and male and female rabbits and the magnitude of decrease was likely correlated to the dose of thyroxine (Fig. 2). On the other hand, N-demethylation of aminopyrine was increased in the female rats, but there was likely no clear correlation between the dose and the magnitude of the increase, but the low dose of thyroxine produced the highest increase (Fig. 2).

Similarly, the low dose of thyroxine produced the maximum increase in the hydroxyla-

FIG. 3. Effect of various doses of thyroxine on hexobarbital hydroxylation by liver microsomes of rats, mice and rabbits.

See the legends for Fig. 2.

FIG. 4. Effect of various doses of thyroxine on aniline hydroxylation by liver microsomes of rats, mice and rabbits.

See the legends for Fig. 2.

tion of hexobarbital in the female rats, but the increase resulted in no significance as increase the dose of thyroxine (Fig. 3). The hydroxylation of hexobarbital was likely relatively stable in the rabbits and only the high dose of thyroxine resulted in a significant decrease and the activity in the mice was decreased by all the doses and there was likely no clear dose response relationship.

The hydroxylation of aniline was increased in the rabbits by the low and middle doses of thyroxine, but high dose of thyroxine did not significantly increased the activity (Fig. 4). The activity in the mice was decreased by all the doses and there was likely no clear dose response relationship (Fig. 4).

The nitroreduction of *p*-nitrobenzoic acid was significantly increased only in the rabbits except the high dose group of female rabbits (Fig. 5). Moreover, the high dose of thyroxine caused a decrease in the nitroreduction in male mice (Fig. 5).

FIG. 5. Effect of various doses of thyroxine on *p*-nitrobenzoic acid nitroreduction by liver microsomes of rats, mice and rabbits. See the legends for Fig. 2.

FIG. 6. Effect of various doses of thyroxine on the activities of NADPH oxidase in liver microsomes of rats, mice and rabbits. See the legends for Fig. 2.

FIG. 7. Effect of various doses of thyroxine on the activities of NADPH-neotetrazolium (NT) reductase in liver microsomes of rats, mice and rabbits. See the legends for Fig. 2.

FIG. 8. Effect of various doses of thyroxine on the activities of NADPH-cyt c reductase in liver microsomes of rats, mice and rabbits. See the legends for Fig. 2.

FIG. 9. Effect of various doses of thyroxine on activities of NADH-cyt c reductase in liver microsomes of rats, mice and rabbits. See the legends for Fig. 2.

6. *Effect of the treatment with various doses of thyroxine on the activities of electron transport systems of liver microsomes in rats, mice and rabbits*

The activity of NADPH oxidase was markedly increased in the rabbits in proportion to the increasing of the dose (Fig. 6). However, the activity in the mice was not increased by any doses. Moreover, in the female rats the activity was increased by all the doses and there was likely no clear dose response relationship (Fig. 6). The activity of NADPH-neotetrazolium reductase was markedly increased in the female rats, male and female rabbits, but there was no clear dose response relationship (Fig. 7). On the other hand, the activity was slightly increased in male rats and there was no clear increase in the high dose group. In contrast, the activity in mice was increased only in the high dose group (Fig. 7). The activity of NADPH-cytochrome c reductase was markedly increased in the rabbits, and there was likely clear dose response relationship (Fig. 8). However, the activity in

FIG. 10. Effect of various doses of thyroxine on the content of cyt P-450 in liver microsomes of rats, mice and rabbits.

See the legends for Fig. 2.

FIG. 11. Effect of various doses of thyroxine on the content of cyt b₅ in liver microsomes of rats, mice and rabbits.

See the legends for Fig. 2.

the mice was not increased by any doses. The activity of NADH-cytochrome c reductase was decreased in all experimental groups with exception of the low dose group of mice (Fig. 9). The amount of cytochrome P-450 was decreased in all animals, according as the increasing dose of thyroxine (Fig. 10). The amount of cytochrome b_5 was not so markedly altered, but the amount was slightly decreased in the high dose group of rats and mice (Fig. 11).

DISCUSSION

The results of present investigation showed that the alterations in the activity of drug-metabolizing enzymes of liver microsomes of mice and rabbits by thyroxine treatment were not similar to those observed in female rats as previously expected (6).

On the other hand, there is no clear sex difference in the alterations of the activity in mice and rabbits as previously expected.

These results indicated that the decreases in the activity of aminopyrine N-demethylation in mice and rabbits and the activity of hexobarbital hydroxylation in mice were not related to interference in the action of androgenic hormone as reported in male rats (3).

The mechanism of these differences in the effect of thyroxine in the rats, mice and rabbits was not clear, but the results given in Figs. 1-5 indicated that those differences are not only due to the dosage of thyroxine, because of the different dose resulted in similar tendency. It is of special interest that the activity of aminopyrine N-demethylation and hexobarbital and aniline hydroxylation is markedly decreased in mice by all doses of thyroxine, notwithstanding the fact that these doses of thyroxine results in only small percentage of the decrease in the body weight.

The alteration in the activity of aminopyrine N-demethylation and hexobarbital hydroxylation in the female rats indicated that the high dose of thyroxine likely abolished the increase in the activity. The following possibility, therefore, could not be excluded that there is dual action of thyroxine on the drug-metabolizing enzyme. The low dose of thyroxine mainly results in the increasing action, but the high dose of thyroxine rather shows the decreasing action.

The different effect of thyroxine, thus, likely due to the differences in the balance of dual action in rats, mice and rabbits and the activities of aminopyrine N-demethylation and hexobarbital hydroxylation in mice and rabbits and the activity of aniline hydroxylation in mice would be sensibly affected by the decreasing action of thyroxine. However, to confirm these interpretations further studies with several different doses of thyroxine will be required.

On the other hand, these differences in the effect of thyroxine on the drug-metabolizing enzymes also are likely related to the alteration in the activity of NADPH-linked electron transport systems. In male and female rabbits the all activities of NADPH oxidase, NADPH-neotetrazolium reductase and NADPH-cytochrome c reductase were markedly increased by the thyroxine treatment and in female rats the all activities were increased, even to less extent. In contrast, in male and female mice the activities of NADPH oxidase,

NADPH-neotetrazolium and NADPH-cytochrome c reductase were not significantly increased with exception of the high dose group of NADPH-neotetrazolium.

The content of cytochrome P-450 was significantly decreased in both sexes of rats, mice and rabbits except the low dose group of female rats and female and male rabbits. These results were in accordance with the observation made by Wada that the content of cytochrome P-450 was decreased in thyroid fed male mice (24). It is also possible to suppose that the content of cytochrome P-450 is likely one of related factors for the decrease in the drug-metabolizing activities in the thyroxine treated animals.

However, at the present time we have only limited information concerning the relationship among the activity of NADPH-cytochrome c reductase and NADPH oxidase, the content of cytochrome P-450 and the drug-metabolizing activity of liver microsomes. Therefore, the conclusive interpretation is not able to be presented at the present time and further studies will be needed.

SUMMARY

The possibility of species difference in the effect of thyroxine administration on the activities of drug-metabolizing enzymes in liver microsomes of both sexes of rats, mice and rabbits was investigated.

The activities of aminopyrine N-demethylation and hexobarbital hydroxylation in male rats were markedly decreased by the thyroxine treatment, but the activities in female rats were slightly increased. In contrast, the activities in both sexes of mice and rabbits were significantly decreased.

On the other hand, the activity of aniline hydroxylation was increased in male and female rats and rabbits, but the activity was decreased in both sexes of mice. The activity of *p*-nitrobenzoic acid reduction was increased in male and female rabbits.

The activities of microsomal electron-transport systems, such as, NADPH oxidase and NADPH-cytochrome c reductase and NADPH-neotetrazolium reductase were increased in both sexes of rats and rabbits by thyroxine. However, the activity of NADH-cytochrome c reductase was markedly decreased in all the groups. The content of cytochrome p-450 was not altered in female rats, but it was decreased in male rats, male and female mice and rabbits.

The results of the present paper showed that the sex difference in the alteration in the activities of aminopyrine N-demethylation and hexobarbital hydroxylation were observed only in rats, but it was not observed in mice and rabbits as expected previously.

Other mechanism is, thus, likely involved in the decrease of the drug-metabolizing activities in mice and rabbits by thyroxine treatment. The content of cytochrome P-450 is likely one of related factors for the decrease in the drug-metabolizing activities in the thyroxine treated animals.

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