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DOSE-DEPENDENT ACTIONS OF THYROXINE ON HEPATIC DRUG METABOLISM IN MALE AND FEMALE RATS

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Abstract—Studies were carried out to compare the effects of various doses of thyroxine (T_4) on hepatic drug metabolism in male and female rats and to evaluate the role of the pituitary gland in the modulation of T_4 action. Administration of small amounts of T_4 (2.5 to 5 μg/100 g body wt/day) to hypophysectomized rats of either sex increased hepatic ethylmorphine demethylase, benzo(a)pyrene hydroxylase and aniline hydroxylase activities. Larger amounts of T_4 (12.5 to 50 μg) reversed the stimulatory effects of the smaller doses. T_4 treatment produced dose-dependent decreases in hepatic cytochrome P-450 content and increases in NADPH–cytochrome *c* reductase activity in hypophysectomized rats of both sexes. Qualitatively similar effects were produced by T_4 administration to thyroidectomized male and female rats. However, larger doses of T_4 were required for maximum stimulation of drug metabolism in thyroidectomized than in hypophysectomized animals. The results indicate that physiological amounts of T_4 uniformly stimulate hepatic drug metabolism in both male and female rats. Supraphysiological amounts, however, inhibit metabolism of some substrates and produce sex differences in T_4 actions. The effects of T_4 are demonstrable in the absence of the pituitary gland but pituitary-dependent factors appear to modulate the magnitude of the response to T_4.

Among the many endocrine factors which influence the activity of hepatic microsomal drug-metabolizing enzymes are the thyroid hormones, thyroxine and tri-iodothyronine [1–8]. Previous investigations have demonstrated that the effects of thyroxine on hepatic mixed function oxidases in rats are dependent upon both the substrate employed and the sex of the experimental animal [1–5]. Administration of thyroxine to male rats decreased the rates of oxidation of those substrates whose metabolism was enhanced by androgen treatment but accelerated the metabolism of other substrates [1]. In female rats, by contrast, thyroxine treatment increased the metabolism of all substrates studied. However, in androgen-treated females, the effects of thyroxine were identical to those in males. On the basis of these and related investigations [4, 5], it has been suggested [2, 5] that thyroxine antagonizes the stimulatory effects of androgens on hepatic microsomal metabolism, but in the absence of androgenic influence, thyroxine increases hepatic mixed function oxidase activity.

In all of the studies cited, extremely large doses of thyroxine (1 to 1.5 mg/kg/day) were employed. Thus, the conclusions reached pertain only to pharmacological and not physiological effects of thyroid hormones. Accordingly, the following studies were carried out to evaluate the actions of physiological amounts of thyroxine on hepatic microsomal drug metabolism and to determine if the effects of small doses, like those of larger amounts, are sex and substrate dependent. In addition, since recent observations [9, 10] indicate that the effects of several hormones on hepatic metabolism are manifested only in the presence of the pituitary gland, the actions of thyroxine in the presence and absence of the pituitary were compared. The results establish that the physiological effects of thyroxine on hepatic mixed function oxidases differ both qualitatively and quantitatively from those produced by larger amounts of hormone and that the effects are demonstrable in the absence of the pituitary gland.

METHODS

Male and female Sprague–Dawley rats, obtained from Zivic–Miller Laboratories, Pittsburgh, PA, were housed under standardized conditions of light (6:00 a.m. to 6:00 p.m.) and temperature (22°). Hypophysectomies and thyroidectomies were performed by the breeder on animals 55 to 60-days-old. All animals were fed Wayne Lab Blox *ad lib.* and those with intact thyroid glands received tap water *ad lib.* to drink. Thyroidectomized rats received 1% $CaCl_2$ in place of water to enhance survival. The $CaCl_2$ did not affect hepatic mixed function oxidases. Na-l-thyroxine (Sigma, St. Louis, MO) in 0.9% NaCl (pH 9.0) was administered as a daily subcutaneous injection between 8:00 and 9:00 a.m. at the doses indicated for 7 days. Controls received vehicle only. Hormonal treatment was initiated 14–21 days after thyroidectomy or hypophysectomy.

All animals were killed by decapitation between 9:00 and 10:00 a.m. Livers were quickly removed and homogenized in 1.15% KCl. Homogenates were centrifuged at 9000 *g* for 20 min and aliquots of the supernatant fraction were removed for enzyme assays or centrifuged in a Beckman preparative ultracentrifuge at 105,000 *g* for 60 min to obtain the microsomes. Microsomal pellets were resuspended in 1.15% KCl containing 0.05 M Tris–HCl buffer (pH 7.4) at a concentration of 4–5 mg

protein/ml. All steps in the preparation of microsomes were performed at 4°.

The demethylation of ethylmorphine and the hydroxylation of aniline were assayed as the amounts of formaldehyde [11] or para-aminophenol [12] formed, respectively, by 1.0 ml hepatic 9000 *g* supernatant fraction (equivalent to 100 mg tissue/ml) incubated with glucose 6-phosphate (9.0 μmoles), NADP (2.1 μmoles), $MgCl_2$ (24.2 μmoles), Tris–HCl buffer (0.05 M, pH 7.4) and ethylmorphine–HCl (12 μmoles) or aniline (6 μmoles) in a final volume of 3.0 ml. Semicarbizide (25 μmoles) served as a trapping agent for formaldehyde produced from ethylmorphine. All incubations were carried out in a Dubnoff metabolic shaking incubator at 37° for 15 min under air. Benzo(a)pyrene hydroxylation was determined by the fluorometric method of Nebert and Gelboin [13]. The incubation medium contained $MgCl_2$ (2.7 μmoles), Tris–HCl buffer (0.05 M, pH 7.4), NADPH (480 nmoles), bovine serum albumin (0.5 mg), and 0.1 ml hepatic 9000 *g* supernatant fraction (50 mg/ml) in a final volume of 1.0 ml. The reaction was initiated by the addition of 100 nmoles benzo(a)pyrene in 50 μl acetone. Samples were incubated at 37° for 8 min under air. Quinine sulfate was calibrated against authentic 3-OH benzo(a)pyrene and routinely used as the fluorescence standard. For all enzyme assays, the appropriate tissue and substrate blanks and standards were carried through the entire procedure. Enzyme activities are presented as the rates of product formation per g of liver, but the effects of thyroxine are identical when activities are expressed per mg of microsomal protein.

Cytochrome P-450 content in isolated microsomes was determined using a Cary 17 recording spectrophotometer at 25° as described by Estabrook *et al.* [14]. NADPH–cytochrome *c* reductase activity was assayed by the method of Phillips and Langdon [15] and microsomal protein was determined by the method of Lowry *et al.* [16]. The statistical significances of differences between group means were determined using Student's *t*-test or the Newman–Keuls multiple range test, as appropriate.

RESULTS

The effects of thyroxine (T_4) administration to thyroidectomized male and female rats on hepatic mixed function oxidases are presented in Tables 1 and 2 respectively. Values obtained from normal animals with intact thyroid glands are also included for comparison.

Small amounts of T_4 (5 or 12.5 μg/100 g) increased body and liver weights in male rats (Table 1) but larger amounts (50–125 μg) did not. All doses of T_4 increased microsomal protein concentration and NADPH–cytochrome *c* reductase activity. Hepatic cytochrome P-450 content was progressively diminished by increasing the dose of T_4. Nonetheless, ethylmorphine (EM) demethylase, benzo(a)pyrene (BP) hydroxylase and aniline hydroxylase activities were significantly increased by T_4 treatment. The metabolism of BP and EM was maximally stimulated by the lower doses of T_4 and began to decline with higher doses. Aniline hydroxylase activity, in contrast, was stimulated by all doses of T_4.

In thyroidectomized female rats (Table 2), T_4 also increased body and liver weights but did not affect microsomal protein content. Cytochrome P-450 concentrations were significantly decreased and NADPH–cytochrome *c* reductase activity was significantly increased by all doses of T_4 studied. The lower doses of T_4 increased EM demethylase and BP hydroxylase activities but larger amounts reversed the effect. The rate of aniline hydroxylation was increased by all doses of T_4.

T_4, when given to hypophysectomized male rats at doses of 5 or 12.5 μg/100 g/day (Table 3), did

Table 1. Effects of thyroxine on hepatic microsomal mixed function oxidases in thyroidectomized male rats*

	Normal male rats	Thyroidectomized male rats			
		Dose of thyroxine (μg/100 g/day)			
		0	5	12.5	50
Body wt (g)	281 ± 6	251 ± 4	284 ± 3†	290 ± 4††	259 ± 6‡,§
Liver wt (g)	10.9 ± 0.4	8.5 ± 0.3	10.4 ± 0.3†	10.8 ± 0.2†	8.9 ± 0.4‡,§
Microsomal protein (mg/g liver)	34.1 ± 1.2	32.6 ± 1.5	37.5 ± 0.8†	39.4 ± 1.0†	38.3 ± 0.5†
Cytochrome P-450 (nmoles/mg protein)	0.83 ± 0.06	0.92 ± 0.07	0.75 ± 0.04†	0.56 ± 0.02†‡	0.43 ± 0.02†,‡
NADPH–cytochrome *c* reductase (nmoles/min/g liver × 10^{-2})	22.2 ± 1.6	9.7 ± 0.9	21.8 ± 1.8†	26.1 ± 1.0†	28.8 ± 2.1†‡
Ethylmorphine demethylase (nmoles/min/g liver)	351.2 ± 32.4	149.7 ± 11.0	320.5 ± 46.6†	326.7 ± 26.7†	278.9 ± 34.5†
Benzo(a)pyrene hydroxylase (nmoles/min/g liver)	24.1 ± 2.1	10.5 ± 1.0	25.9 ± 2.9†	23.5 ± 1.6†	15.8 ± 1.3‡
Aniline hydroxylase (nmoles/min/g liver)	37.3 ± 2.5	19.2 ± 2.6	34.0 ± 2.1†	38.6 ± 2.6†	43.5 ± 3.2†

*Values are expressed as mean ± S. E. M.; there were seven to ten animals per group.
†$P < 0.05$ vs 0 μg T_4 group.
‡$P < 0.05$ vs 5 μg T_4 group.
§$P < 0.05$ vs 12.5 μg T_4 group.

Table 2. Effects of thyroxine on hepatic microsomal mixed function oxidases in thyroidectomized female rats*

	Normal female rats	Thyroidectomized female rats			
		Dose of thyroxine (μg/100 g/day)			
		0	5	12.5	50
Body wt (g)	231 ± 6	220 ± 4	235 ± 4†	232 ± 3†	238 ± 3†
Liver wt (g)	8.7 ± 0.3	7.5 ± 0.4	8.8 ± 0.3†	8.6 ± 0.5	9.4 ± 0.4†
Microsomal protein (mg/g liver)	28.9 ± 0.9	30.1 ± 0.6	29.8 ± 0.8	32.0 ± 0.8	30.5 ± 0.8
Cytochrome P-450 (nmoles/mg protein)	0.61 ± 0.03	0.66 ± 0.02	0.57 ± 0.02†	0.57 ± 0.02†	0.45 ± 0.02†,‡,§
NADPH-cytochrome *c* reductase (nmoles/min/g liver × 10^{-2})	19.6 ± 1.3	10.3 ± 0.5	23.8 ± 1.6†	26.5 ± 0.8†	26.2 ± 0.8†
Ethylmorphine demethylase (nmoles/min/g liver)	118.7 ± 4.2	80.0 ± 11.1	120.6 ± 7.2†	129.7 ± 3.9†	103.3 ± 6.0§
Benzo(a)pyrene hydroxylase (nmoles/min/g liver)	4.1 ± 0.3	3.4 ± 0.3	4.4 ± 0.3†	4.3 ± 0.3†	2.5 ± 0.2†,‡,§
Aniline hydroxylase (nmoles/min/g liver)	31.2 ± 0.9	5.3 ± 0.6	10.5 ± 1.0†	13.4 ± 0.4†	11.7 ± 1.3†

*Values are expressed as mean ± S. E. M.; there were seven to ten animals per group.
†$P < 0.05$ vs 0 μg T_4 group.
‡$P < 0.05$ vs 5 μg T_4 group.
§$P < 0.05$ vs 12.5 μg T_4 group.

Table 3. Effects of thyroxine on hepatic microsomal mixed function oxidases in hypophysectomized male rats*

	Dose of thyroxine (μg/100 g/day)		
	0	5	12.5
Body wt (g)	179 ± 4	172 ± 4	166 ± 3†
Liver wt (g)	5.9 ± 0.2	5.2 ± 0.1†	5.2 ± 0.1†
Microsomal protein (mg/g liver)	33.8 ± 1.2	35.8 ± 1.3	33.3 ± 1.0
Cytochrome P-450 (nmoles/mg protein)	0.78 ± 0.05	0.50 ± 0.03†	0.38 ± 0.02†,‡
NADPH–cytochrome *c* reductase (nmoles/min/g liver × 10^{-2})	11.9 ± 0.5	24.1 ± 1.6†	24.6 ± 1.4†
Ethylmorphine demethylase (nmoles/min/g liver)	108.4 ± 6.2	178.6 ± 15.1†	140.6 ± 14.2†
Benzo(a)pyrene hydroxylase (nmoles/min/g liver)	7.6 ± 0.4	10.2 ± 0.7†	7.9 ± 0.9‡
Aniline hydroxylase (nmoles/min/g liver)	8.0 ± 0.8	20.7 ± 1.7†	15.1 ± 1.3†

*Values are expressed as mean ± S. E. M.; there were were nine to ten animals per group.
†$P < 0.05$ vs 0 μg T_4 group.
‡$P < 0.05$ vs 5 μg T_4 group.

not affect hepatic microsomal protein content. However, T_4 significantly reduced cytochrome P-450 concentrations and increased NADPH–cytochrome *c* reductase activity at both doses. Ethylmorphine demethylase, aniline hydroxylase and benzo(a)pyrene hydroxylase activities were maximally stimulated by the 5 μg dose of T_4. The higher dose of T_4 produced a smaller increment in aniline hydroxylase activity and had no significant effects on the rates of ethylmorphine demethylation or benzo(a)pyrene hydroxylation. Still larger amounts of T_4 (25–50 μg/100 g/day) produced further decrements in hepatic microsomal cytochrome P-450 content and diminished the rates of BP hydroxylation, EM demethylation and aniline hydroxylation to below control values. However, the higher doses were somewhat toxic when given to hypophysectomized rats and the physical condition of most animals rapidly deteriorated. A lower dose of T_4 (2.5 μg/100 g/day) than presented in Table 3 increased the rates of BP, EM and aniline metabolism and decreased cytochrome P-450 content, but to a lesser extent than the 5 μg dose.

In hypophysectomized female rats (Table 4), thyroxine produced effects essentially identical to those in males. Small amounts of thyroxine (2.5 to 5 μg) increased the metabolism of EM, BP and aniline; maximum stimulation occurred with the 5 μg dose. Larger doses (12.5 to 50 μg) progressively reversed the stimulatory effects of smaller amounts. As noted above for hypophysectomized

Table 4. Effects of thyroxine on hepatic microsomal mixed function oxidases in hypophysectomized female rats*

	Dose of thyroxine (μg/100 g/day)		
	0	5	12.5
Body wt (g)	181 ± 2	174 ± 3†	171 ± 3†
Liver wt (g)	6.4 ± 0.1	5.7 ± 0.1†	5.9 ± 0.3
Microsomal protein (mg/g liver)	32.2 ± 1.0	35.3 ± 0.5	32.9 ± 1.4
Cytochrome P-450 (nmoles/mg protein)	0.62 ± 0.04	0.40 ± 0.02†	0.26 ± 0.02†,‡
NADPH–cytochrome *c* reductase (nmoles/min/g liver × 10^{-2})	6.3 ± 0.3	13.8 ± 0.9†	16.1 ± 1.3†
Ethylmorphine demethylase (nmoles/min/g liver)	140.0 ± 12.2	266.7 ± 22.1†	199.3 ± 23.7‡
Benzo(a)pyrene hydroxylase (nmoles/min/g liver)	4.7 ± 0.6	6.7 ± 0.6†	3.8 ± 0.3‡
Aniline hydroxylase (nmoles/min/g liver)	13.6 ± 1.3	37.0 ± 3.1†	29.0 ± 2.2‡

*Values are expressed as mean ± S. E. M.; there were eight animals per group.
†$P < 0.05$ vs 0 μg T_4 group.
‡$P < 0.05$ vs 5 μg T_4 group.

male rats, doses of T_4 above 12.5 μg produced signs of toxicity in hypophysectomized females. For all doses studied, T_4 produced dose-dependent decreases in cytochrome P-450 concentrations and increases in NADPH–cytochrome *c* reductase activity.

DISCUSSION

The results of previous studies, in which very large amounts of thyroxine were given to rats, have led various investigators to conclude that the actions of the hormone on hepatic mixed function oxidases are sex and substrate dependent [1–5]. However, our observations indicate that the same conclusions are not applicable to the physiological effects of thyroxine. In both male and female rats, small amounts of thyroxine increased mixed function oxidase activity irrespective of the substrate employed. Metabolism of both androgen-dependent (ethylmorphine and benzo(a)pyrene) and androgen-independent (aniline) substrates was accelerated by thyroxine treatment. Thus, under physiological conditions, thyroxine uniformly stimulates hepatic drug metabolism.

Despite the differences in the amount of hormone administered, our findings tend to confirm some of the conclusions of other investigators [3–5]. For example, as the dose of thyroxine was increased well beyond the physiological range, the stimulatory effects were diminished and, in some cases, inhibition of drug metabolism resulted. The tendency toward inhibition of metabolism with larger amounts of thyroxine was greatest for androgen-dependent substrates (benzo(a)pyrene and ethylmorphine) as previously noted by Kato *et al.* [3–5]. However, under normal circumstances, the stimulatory effects are clearly manifested since removal of the thyroid gland has consistently been found to diminish the rates of drug metabolism [2, 3, 5]. Furthermore, in a number of clinical investigations [17–20] hypothyroidism has been shown to increase the plasma half-life of drugs whereas hyperthyroidism accelerates drug clearance.

The mechanism(s) responsible for the dose-dependent effects of thyroxine on hepatic drug metabolism in rats probably involves cytochrome(s) P-450. Even at the lowest doses of thyroxine studied, significant decreases in hepatic cytochrome P-450 content were found. However, since the concentration of cytochrome P-450 is normally not rate-limiting for hepatic drug metabolism, small changes in microsomal hemeprotein content should not compromise the capacity for oxidative metabolism. At low doses of thyroxine, therefore, the effect of the hormone to increase NADPH–cytochrome *c* reductase activity, insofar as it may reflect changes in the activity of NADPH–cytochrome P-450 reductase, probably accounts for the stimulation of drug metabolism. With higher doses of thyroxine and progressively diminishing amounts of hepatic cytochrome P-450, however, the concentration of cytochrome may become rate-limiting and ultimately produce a decline in the rates of drug metabolism. Selective effects on certain species of cytochrome P-450 could also account for the substrate-dependent inhibition of drug metabolism by large amounts of thyroxine.

Recent studies have demonstrated an important role for the pituitary gland in the modulation of hormonal effects on hepatic mixed function oxidases. The actions of testosterone and estradiol, for example, are not demonstrable in hypophysectomized rats [9, 10] and growth hormone exerts opposite effects in the presence and absence of the pituitary [21], suggesting mediation by or interaction with pituitary-dependent factors. The actions of thyroxine, in contrast, are qualitatively identical in thyroidectomized and hypophysectomized animals. Thus, thyroxine is able to act directly on the liver, independent of other hormonal influences. However, the sensitivity of rats to the effects of thyroxine was affected by the pituitary gland. Smaller doses of hormone were

required for maximum stimulation of drug metabolism in hypophysectomized than in thyroidectomized rats of either sex. Furthermore, in male rats, the stimulatory effects of thyroxine on hepatic mixed function oxidases were greater in the presence than in the absence of the pituitary gland, whereas in females the opposite occurred. These observations suggest at least some degree of interaction between thyroxine and pituitary-dependent factors. Further studies are now needed to determine the nature of such interactions and their significance in the overall regulation of hepatic drug metabolism.

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