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Modulation of thyroid hormone actions on hepatic drug metabolism by androgens and estrogens

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Numerous studies have established that the thyroid hormones thyroxine and tri-iodothyronine participate in the regulation of hepatic microsomal drug-metabolizing enzymes [1–10]. The results of most investigations indicate that thyroidectomy decreases hepatic monooxygenase activities. However, varying effects of thyroid hormone administration on drug metabolism have been reported. The divergent results may be attributable, at least in part, to differences in the doses of thyroid hormones used. Kato and coworkers [1–4] gave very large doses of thyroxine to rats and found that the effects on drug metabolism were sex and substrate dependent. Administration of thyroxine to female rats increased the rates of metabolism of all substrates examined. In male rats, by contrast, Kato *et al.* [1–4] found that thyroxine decreased the rates of metabolism of substrates whose oxidation was stimulated by androgens but increased the metabolism of androgen-independent substrates. The investigators concluded that thyroxine antagonized the stimulatory effects of testosterone on hepatic drug metabolism but, in the absence of testosterone or with androgen-independent substrates, thyroxine increased the rates of drug metabolism. We subsequently suggested that the conclusions of Kato and coworkers were applicable only to supraphysiological doses of thyroxine since physiological amounts of hormone uniformly stimulated microsomal drug metabolism in male and female rats [8]. However, Skett and Weir [10] have proposed recently that there are sex differences in the actions of even physiological amounts of thyroxine on hepatic drug metabolism. To further pursue this issue, the following studies were done to evaluate the role of the gonadal hormones (testosterone and estradiol) in modulating the effects of thyroxine on drug metabolism.

Methods

Male 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°). Castrations 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 metabolism of ethylmorphine, aniline, or benzo[*a*]pyrene. Sodium-l-thyroxine (Sigma Chemical Co., 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 a dose of 25 μg/kg body wt for 7 days. Testosterone (Testosterone cypionate; Upjohn Co., Kalamazoo, MI; 5 mg/100 g body wt) and estradiol (Estradiol cypionate; Upjohn Co.; 10μg/100 g body wt) were administered as single subcutaneous depot injections. We previously found that those doses maintained normal sex ancessory tissue weights in castrated male and female rats, respectively, for at least 4 weeks. Hormonal treatment was initiated 14 days after thyroidectomy and/or castration.

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 the supernatant fraction was 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.

The demethylation of ethylmorphine and the hydroxylation of aniline were assayed as the amounts of formaldehyde [11] or *p*-aminophenol [12] formed, respectively, by hepatic microsomes, as described previously [8]. Semicarbizide (25 μmoles) served as a trapping agent for formaldehyde produced from ethylmorphine. 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]. 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.

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 the Newman–Keuls multiple range test [17].

Results and discussion

As previously reported [8], thyroidectomy decreased the rates of metabolism of androgen-dependent (ethylmorphine, benzo[*a*]pyrene) as well as androgen-independent (aniline) substrates by rat liver microsomes (Table 1). Thyroidectomy also decreased NADPH-cytochrome *c* reductase activity but did not significantly affect microsomal cytochrome P-450 levels. In none of the experiments described do the effects of thyroid hormones on drug metabolism correlate closely with changes in cytochrome P-450 content. It is possible that cytochrome P-450 levels are not rate-limiting for the monooxygenase reactions studied or alternately that thyroid hormones effect changes in specific isoenzymes of cytochrome P-450 which are not reflected by total cytochrome P-450 concentrations. In most cases (Tables 1 and 2), the effects of thyroxine on microsomal drug metabolism correlated closely with changes in NADPH-cytochrome *c* reductase activity, as reported previously [8].

Administration of thyroxine (T_4; 25 μg/kg body wt) to thyroidectomized rats reversed the effects of thyroidectomy on microsomal metabolism (Table 1). Castration further decreased the rates of metabolism of the androgen-dependent substrates, ethylmorphine (EM) and benzo[*a*]pyrene (BP), in thyroidectomized rats but did not further affect aniline hydroxylase activity, NADPH-cytochrome *c* reductase activity, or cytochrome P-450 levels. T_4 administration to thyroidectomized-castrated rats produced changes in microsomal metabolism very similar to those produced in thyroidectomized rats with intact testes. EM demethylase, BP hydroxylase, aniline hydroxylase, and NADPH-cytochrome *c* reductase activities were increased by T_4, and cytochrome P-450 concentrations declined (Table 1). Administration of testosterone to thyroidectomized-castrated rats restored microsomal metabolism to levels comparable to those in thyroidectomized rats with intact testes. Administration of both T_4 and testosterone

Table 1. Effects of thyroxine (T_4) and testosterone on hepatic oxidative metabolism in thyroidectomized (Thyrex) and thyroidectomized-castrated male rats*

	Body wt (g)	Liver wt (g)	Microsomal protein (mg/g liver)	Ethylmorphine metabolism (nmoles/ min × mg protein)	Aniline metabolism (nmoles/ min × mg protein) (10^{-1})	Benzo [*a*]pyrene metabolism (nmoles/ min × mg protein) (10^{-1})	NADPH-cytochrome *c* reductase (nmoles/ min × mg protein)	Cytochrome P-450 (nmoles/mg protein)
Control	331 ± 7	13.2 ± 0.6	30.8 ± 3.0	10.9 ± 0.9	9.1 ± 0.9	6.4 ± 0.6	91.9 ± 8.6	0.74 ± 0.06
Thyrex	220 ± 4†	7.9 ± 0.3†	33.3 ± 1.5	4.8 ± 0.3†	3.5 ± 0.3†	1.9 ± 0.2†	30.6 ± 1.6†	0.80 ± 0.04
Thyrex + T_4	257 ± 3†‡	9.9 ± 0.2†‡	35.1 ± 1.1	9.4 ± 0.6‡	8.2 ± 0.8‡	5.3 ± 0.3‡	76.6 ± 2.5‡	0.52 ± 0.03†‡
Thyrex + castrated	195 ± 4†‡	6.0 ± 0.2†‡	32.5 ± 0.8	2.9 ± 0.2†‡	3.2 ± 0.3†	1.0 ± 0.2†	29.9 ± 1.9†	0.64 ± 0.03†‡
Thyrex + castrated + T_4	232 ± 4†§	9.5 ± 0.4†‡§	34.3 ± 1.5	6.6 ± 0.5†‡§	6.2 ± 0.6‡§	2.6 ± 0.3†§	66.8 ± 4.2‡§	0.44 ± 0.02†‡§
Thyrex + castrated + testosterone	230 ± 4†§	9.3 ± 0.6†§	31.9 ± 1.6	5.7 ± 0.4†§	4.1 ± 0.5†	2.2 ± 0.2†§	38.6 ± 3.6†§	0.78 ± 0.06§
Thyrex + castrated + testosterone + T_4	269 ± 6†‡§‖	10.1 ± 0.6†‡§	32.4 ± 1.3	10.1 ± 0.9‡§‖	9.0 ± 0.9‡§‖	5.7 ± 0.4‡§‖	83.6 ± 7.0‡§‖	0.75 ± 0.08§

* Values are expressed as means ± S.E.M.; six to eight animals per group.
† $P < 0.05$ vs control group.
‡ $P < 0.05$ vs Thyrex group.
§ $P < 0.05$ vs Thyrex + castrated group.
‖ $P < 0.05$ vs Thyrex + castrated + testosterone group.

Table 2. Effects of thyroxine (T_4) and estradiol (E_2) on hepatic oxidative metabolism in thyroidectomized (Thyrex)-castrated male rats*

	Body wt (g)	Liver wt (g)	Microsomal protein (mg/g liver)	Ethylmorphine metabolism (nmoles/ min × mg protein)	Aniline metabolism (nmoles/ min × mg protein) (10^{-1})	Benzo[*a*]pyrene metabolism (nmoles/ min × mg protein) (10^{-1})	NADPH-cytochrome *c* reductase (nmoles/ min × mg protein)	Cytochrome P-450 (nmoles/mg protein)
Thyrex + castrated	205 ± 8	7.2 ± 0.6	29.5 ± 1.2	3.0 ± 0.3	3.3 ± 0.4	1.1 ± 0.2	41.4 ± 3.9	0.72 ± 0.05
Thyrex + castrated + T_4	238 ± 12†	8.9 ± 0.4†	34.3 ± 0.5†	5.1 ± 0.5†	7.8 ± 0.5†	2.3 ± 0.2†	86.9 ± 6.3†	0.58 ± 0.06
Thyrex + castrated + E_2	198 ± 7‡	7.1 ± 0.5‡	29.7 ± 0.5‡	2.4 ± 0.3‡	2.6 ± 0.3‡	0.7 ± 0.1‡	39.7 ± 3.6‡	0.72 ± 0.03
Thyrex + castrated + T_4 + E_2	195 ± 8‡	7.5 ± 0.5‡	32.7 ± 1.0	3.3 ± 0.2‡§	4.4 ± 0.4‡§	1.4 ± 0.1‡§	75.8 ± 3.7†§	0.48 ± 0.04†§

* Values are expressed as means ± S.E.M.; seven animals per group.
† $P < 0.05$ vs Thyrex-castrated group.
‡ $P < 0.05$ vs Thyrex-castrated + T_4 group.
§ $P < 0.05$ vs Thyrex-castrated + E_2 group.

to thyroidectomized-castrated animals further increased enzyme activities to levels found in the control group (Table 1). Thus, whether or not testosterone was present, T_4 treatment increased the rates of hepatic drug metabolism.

The effects of estradiol (E_2) on the response of thyroidectomized-castrated male rats to T_4 are shown in Table 2. We and others previously reported that E_2 administration to castrated male rats decreases the rates of microsomal drug metabolism and lowers cytochrome P-450 concentrations [18]. However, in animals that were thyroidectomized as well as castrated, E_2 did not significantly affect microsomal metabolism (Table 2). The absence of E_2 effects is probably attributable, at least in part, to the already very low rates of metabolism in thyroidectomized-castrated rats. T_4 administration to E_2-treated thyroidectomized-castrated males increased EM demethylase, aniline hydroxylase, BP hydroxylase, and NADPH-cytochrome *c* reductase activities, but the stimulatory effects of T_4 were smaller than those produced in thyroidectomized-castrated animals not receiving E_2 (Table 2). Thus, E_2 appeared to modulate the magnitude of the response to T_4 but the effects of T_4 were qualitatively similar in the presence and absence of E_2.

The results indicate that physiological amounts of T_4 uniformly stimulate hepatic microsomal drug metabolism in male rats regardless of the gonadal hormone status of the animals. These observations are consistent with our previous findings, indicating that T_4 increases the rates of microsomal metabolism in male and female rats [8]. The differences between our observations and those of Kato and coworkers [1–4] are probably attributable to the differences in the doses of T_4 employed. We have also found, like Kato *et al.*, that the effects of very high doses of T_4 are indeed sex and substrate dependent. However, the effects of physiological amounts of T_4 are not qualitatively affected by sex or gonadal hormones. Under normal circumstances, the stimulatory effects of T_4 on drug metabolism must be manifested since removal of the thyroid gland decreases the rates of microsomal metabolism in both male and female rats [1, 4, 8]. Skett and Weir [10] recently suggested that the effects of even physiological amounts of T_4 are sex and substrate dependent. However, the latter investigators studied the metabolism of different substrates than those used by us [8] or by Kato and coworkers [1–4] in earlier studies. Therefore, it is possible that some of the effects of thyroid hormones in drug metabolism are substrate-specific. In addition, some of the results of Skett and Weir [10] are difficult to interpret. For example, in some instances they found that thyroidectomy and T_4 administration produced the same effects on microsomal metabolism. A physiological replacement dose of T_4 should prevent or reverse the effects of thyroidectomy, not potentiate them. The authors did not offer any explanation for those apparently discrepant findings. The stimulatory effects of T_4 on drug metabolism which we have consistently observed are also supported by various clinical studies demonstrating that the clearance of drugs from plasma is accelerated in hyperthyroid patients and prolonged with hypothyroidism [19–22].

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