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Thyroid hormone regulation of heme oxidation in the liver

(triiodothyronine/heme oxygenase/cytochrome P-450)

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ABSTRACT **The effects of 3,5,3′-triiodothyronine (T3) on heme oxygenase (EC 1.14.99.3) activity and cytochrome P-450 content in liver were examined in thyroidectomized rats. T3, when administered for 5 days at a dose of 6 μg/100 g of body weight, stimulated basal heme oxygenase activity ≈2-fold compared to diluent-treated animals. The induction of heme oxygenase by cobalt heme also was enhanced ≈3-fold in T3-treated animals. T3 treatment lowered cytochrome P-450 content by ≈50% and potentiated the depletion of this heme protein after cobalt heme administration. Reverse T3 had no effect either on cytochrome P-450 content or on heme oxygenase activity in liver. The time course of response to a single dose of T3 (50 μg/100 g of body weight) revealed that both basal and cobalt heme-induced heme oxygenase activity peaked at 48 hr and that cytochrome P-450 content declined to ≈40% of controls at 96 hr. Examination of microsomal proteins by polyacrylamide gel electrophoresis after T3 treatment disclosed that major bands in the M_r ≈50,000–55,000 region were diminished. The administration of T3 together with SKF-525A, a compound known to complex with the heme prosthetic group of cytochrome P-450, resulted in partial preservation of these proteins. These data indicate that thyroid hormone can regulate heme oxygenase activity and concomitantly can lower cytochrome P-450 content in liver. The hormone also can act in a synergistic fashion to enhance the response of hepatic heme oxygenase to a chemical inducer of the enzyme. Thyroid status thus may be a potentially significant determinant of the rate of heme oxidation in the liver.**

The mammalian liver is a major target organ for the action of many endocrine substances (1), including thyroid hormone (2–4). This hormone has been shown to regulate diverse hepatic functions such as serum protein synthesis (5), bile flow and composition (6, 7), lipid metabolism (8), and transmembrane sodium transport (9, 10). The thyroid status of animals (11) and humans (12) also influences cytochrome P-450-dependent drug metabolism and large amounts of thyroid hormone have been shown to decrease the content of this heme protein in the liver (11, 13–16). However, in none of these studies was the time course or stereospecificity of these hormone effects examined and in most, very large doses of thyroid hormone were used to demonstrate changes in cytochrome P-450 content.

Previous studies from this laboratory have shown that natural steroids regulate the rate of hepatic porphyrin-heme biosynthesis and cytochrome P-450 formation (17–19) and that insulin, thyroid hormone, and hydrocortisone play a permissive role in the steroid and drug induction of the rate-limiting enzyme of heme formation, δ-aminolevulinate synthase, in chicken embryo liver cell cultures (19, 20).

In this study, we examined in the rat the effects of 3,5,3′-triiodothyronine (T3) on the basal and cobalt protoporphyrin IX (cobalt heme)-induced activity of heme oxygenase (EC 1.14.99.3) (21), the rate-limiting enzyme of heme degradation, and on cytochrome P-450 content in liver. T3 administration led to an elevation of basal heme oxygenase activity and an enhancement in the extent of heme oxygenase induction by cobalt heme. The increases in this enzyme activity were accompanied by decreases in hepatic cytochrome P-450 content. Thyroid status thus may be a potentially significant determinant of the endocrine milieu that conditions the adaptive responses of the liver to chemicals that perturb heme and cytochrome P-450 metabolism.

MATERIALS AND METHODS

Materials. T3 was purchased from Sigma and 3,3′,5-triiodothyronine (rT3) was purchased from Calbiochem–Behring. SKF-525A was a generous gift of Smith Kline & French. Cobalt heme was purchased from Porphyrin Products (Logan, UT). All other chemicals were of the highest purity commercially available.

Animal Preparation. Thyroidectomized or sham-operated male Sprague–Dawley rats (130–170 g) were purchased from Charles River Breeding Laboratories (Wilmington, MA). Intact male rats of the same strain (150–200 g) were purchased from Taconic Farms (Germantown, NY) and were maintained on standard rat chow and drinking water ad lib. Thyroidectomized animals were maintained on a low iodine, Remington Diet (Teklad, Madison, WI) with 0.9% $CaCl_2$ in drinking water ad lib to enhance survival. T3, rT3 dissolved in 1 mM NaOH, or the diluent alone was administered intraperitoneally as indicated in the legends of the figures. Sixteen hours prior to killing, isotonic saline or cobalt heme (12.5 μmol/kg of body weight) was administered subcutaneously and animals were starved but allowed free access to water. This dose of cobalt heme was given because it has been found to suboptimally induce heme oxygenase and thus could be used to determine whether synergism of the enzyme induction response with T3 administration existed. Intact animals received T3 (15 μg/100 g of body weight) or SKF-525A (5 mg/100 g of body weight) (or both) dissolved in isotonic saline or diluent daily for 4 days and were killed on the 5th day, 24 hr after the final injections. Animals were killed by decapitation and the livers were perfused *in situ* with ice-cold isotonic saline until blanched.

Livers were removed, minced, and homogenized in 0.1 M potassium phosphate buffer (pH 7.4) containing 0.25 M sucrose [the tissue buffer ratio was 1:3 (wt/vol)] as described (22). Briefly, the homogenate was centrifuged for 10 min at 10,000 × g at 4°C; the supernatant was removed and centrifuged for 1 hr at 100,000 × g at 4°C in a Beckman L5-50 ultracentrifuge. The resultant microsomal pellet was washed and resuspended in a small volume of 0.1 M potassium phosphate buffer (pH 7.4). Plasma was collected at the time of death and assayed for serum thyrotropin (TSH) by radioimmunoassay.

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Abbreviations: T3, 3,5,3′-triiodothyronine; rT3, 3,3′,5-triiodothyronine; TSH, thyrotropin; cobalt heme, cobalt protoporphyrin IX.

Enzyme Assays. The microsomal suspension was assayed for heme oxygenase activity as described (22). The concentration of bilirubin produced was calculated by using an extinction coefficient of 40 $mM^{-1} \cdot cm^{-1}$ between 464 and 530 nm. Cytochrome P-450 content was measured by the method of Omura and Sato (23) with sodium dithionite as a reducing agent. All spectral studies were carried out by using an Aminco DW2A spectrophotometer in the split-beam mode. Protein concentrations were measured by the method of Lowry *et al.* (24) with bovine serum albumin as a standard.

Electrophoresis. Polyacrylamide gel electrophoresis of microsomal proteins was carried out by the method of Weber and Osborn (25). Aliquots of 15 μg of protein were layered on stacking gels and the gels were run at 4°C over ≈4–5 hr.

Statistics. Statistical significance was determined by using an unpaired Student's t test. A value of $P < 0.05$ was considered significant.

RESULTS

Effect of Surgical Thyroidectomy and Low Iodine Diet on Growth. After thyroidectomy, animals maintained on a low iodine diet for a minimum of 7 wk weighed less than one-half their sham-operated littermates which were maintained on standard rodent chow (190 ± 5 g, n = 42 vs. 438 ± 8 g, n = 15; mean ± SEM). Hypothyroidism was confirmed biochemically by a pronounced elevation in serum TSH, a biochemical hallmark of primary hypothyroidism.

Effect of T3 on Heme Oxygenase Activity in Liver. T3 administration (6 μg/100 g of body weight) for 5 days to thyroidectomized rats produced a dose-dependent increase in both basal and cobalt heme-inducible heme oxygenase activity (Fig. 1); an ≈2-fold increase in basal and an ≈3-fold increase in inducible enzyme activity was produced by this treatment. At a lower dose (0.3 μg/100 g of body weight), one which renders thyroidectomized rats euthyroid (26), an intermediate level of both basal and induced enzyme activity was achieved. Results from the lower dose of T3 were similar to those observed in the sham-operated animals [basal heme oxygenase activity, 2.49 ± 0.25 nmol of bilirubin/mg of protein/hr, and cobalt heme-stimulated heme oxygenase activity, 5.85 ± 0.36 nmol of bilirubin/mg of protein/hr (mean ± SEM)], indicating that this dose of T3 normalized these enzymic parameters.

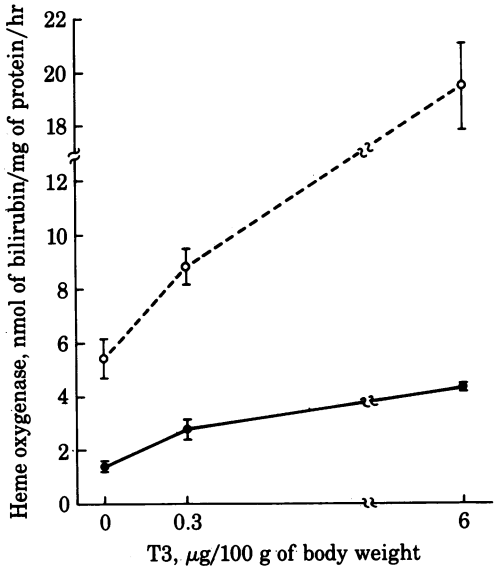

FIG. 1. Effect of T3 on hepatic heme oxygenase activity. Thyroidectomized animals were treated for 5 days with the daily dose of T3 indicated on the abscissa given as two divided doses. Sixteen hours prior to killing, animals received either cobalt heme (12.5 μmol/kg of body weight) (○---○) or an equivalent volume of isotonic saline (●——●). Each datum point represents the mean ± SEM of three animals.

Effects of T3 and rT3 on Heme Oxygenase Activity and Cytochrome P-450 Content in Liver. The stereospecificity of the effect of T3 on heme oxygenase activity was examined by administering to thyroidectomized rats for 5 days either T3 or rT3, a biologically impotent analogue, at the dose of 6 μg/100 g of body weight. This dose of T3 is known to quickly render rats hyperthyroid (27). Both basal and cobalt heme-induced enzyme activities were significantly elevated in the T3-treated animals, whereas the rT3-treated group was indistinguishable from the control group (Fig. 2). There was a concomitant decrease in cytochrome P-450 content in the T3-treated animals. Hypothyroid animals receiving cobalt heme (12.5 μmol/kg of body weight) 16 hr prior to killing exhibited an ≈2.6-fold increase in heme oxygenase and an ≈25% decrease in hepatic cytochrome P-450 content. Animals receiving T3 alone had an ≈2-fold increase in heme oxygenase activity and an ≈50% decrease in cytochrome P-450 content. The administration of both T3 and cobalt heme resulted in an ≈8-fold increase in heme oxy-

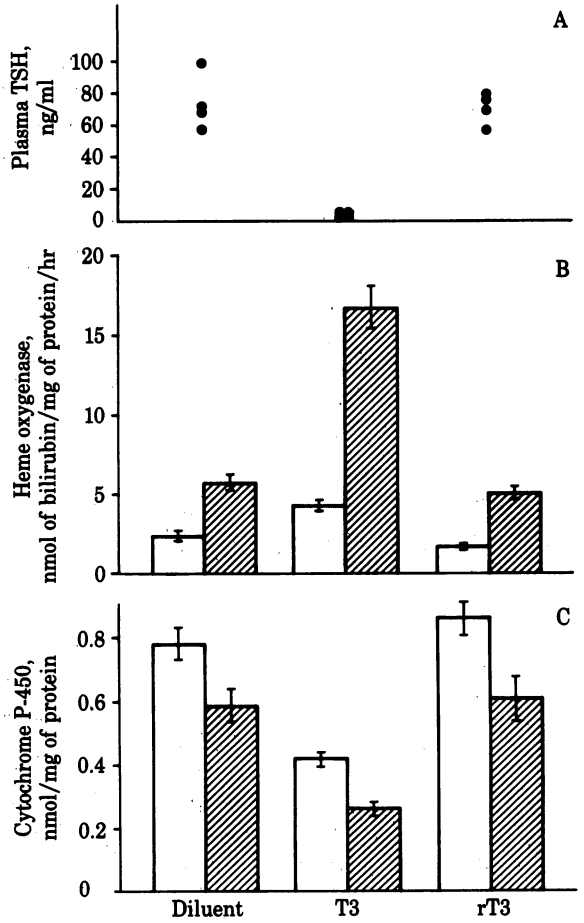

FIG. 2. Effect of T3 and rT3 on plasma TSH concentration (*A*), hepatic heme oxygenase activity (*B*), and cytochrome P-450 content (*C*). Thyroidectomized animals were treated as described in the legend to Fig. 1. (*A*) Plasma TSH concentration at the time of killing in each treatment group. Each datum point represents a single animal treated as indicated on the abscissa. (*B*) Heme oxygenase activity in isotonic saline-treated (open bars) or cobalt heme-treated (hatched bars) animals. (*C*) Cytochrome P-450 content in the animals. Each datum point in *B* and *C* represents the mean ± SEM of at least four animals.

genase and an ≈67% depletion of the heme protein compared with diluent-treated animals. When compared with T3-treated animals, those receiving both the hormone and cobalt heme exhibited an ≈4-fold increase in enzyme activity and an ≈38% decrease in the content of cytochrome P-450. The activities of ethylmorphine demethylase and aniline hydroxylase paralleled the changes in hepatic cytochrome P-450 content (data not shown). T3 added *in vitro* had no effect on cytochrome P-450 content or on heme oxygenase activity. Fig. 2A demonstrates that, whereas rT3 had no effect on the elevated serum levels of TSH in the thyroidectomized animals, T3 effectively suppressed levels below detectability, as expected.

Time Course of Action of a Single Dose of T3 on Heme Oxygenase Activity and Cytochrome P-450 Content in Liver. A single injection of T3 (50 μg/100 of body weight) stimulated both basal and cobalt heme-inducible heme oxygenase in thyroidectomized rats. The time course of this response over 96 hr (Fig. 3) was similar to the response of TSH. There was an

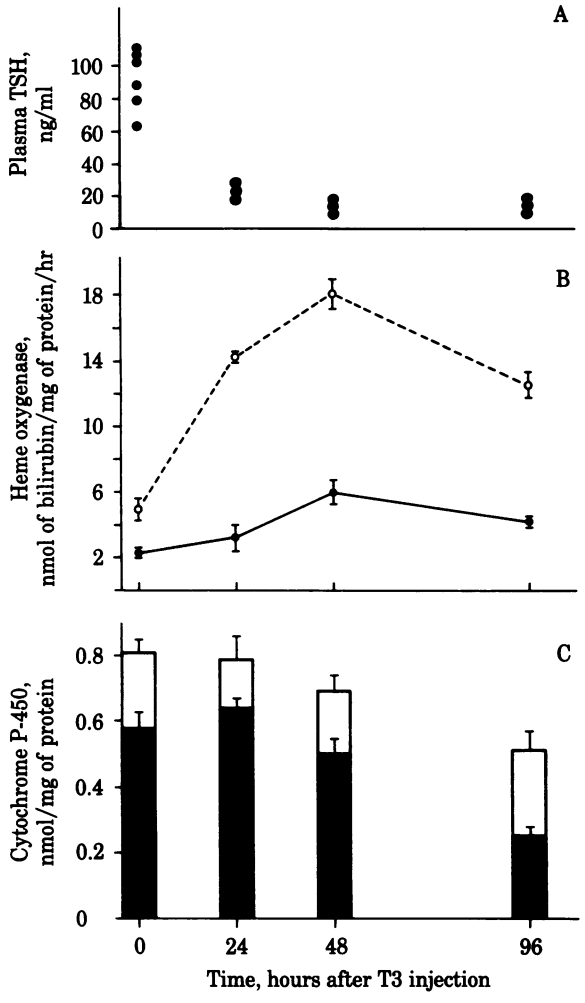

FIG. 3. Time course of the effect of a single dose of T3 on plasma TSH concentration (*A*), hepatic heme oxygenase activity (*B*), and cytochrome P-450 content (*C*). At time 0 animals received T3 at a dose of 50 μg/100 g of body weight. (Time 0 control animals received diluent.) Animals were killed at the times indicated on the abscissa. Each animal received cobalt heme (12.5 μmol/kg of body weight) or saline 16 hr prior to killing. (*A*) Plasma TSH concentration in individual animals. (*B*) Hepatic heme oxygenase activity in cobalt heme-treated (○---○) or saline-treated (●——●) animals. (*C*) Cytochrome P-450 content in cobalt heme-treated (solid bars) or saline-treated (open bars) animals. Each datum point in *B* and *C* represents the mean ± SEM of three to six animals.

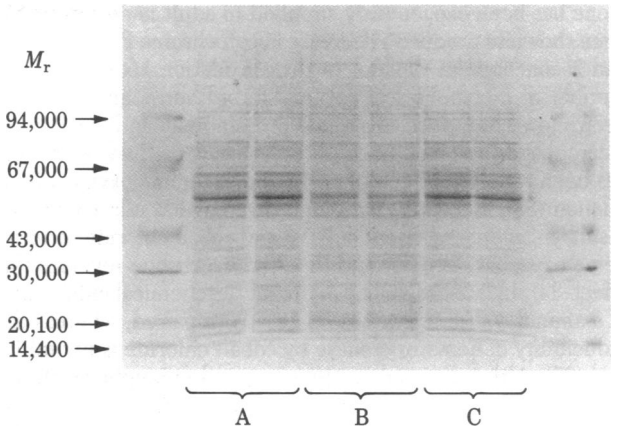

FIG. 4. $NaDodSO_4$/polyacrylamide gel electrophoresis of hepatic microsomal proteins from thyroidectomized animals treated with diluent (*A*), T3 (*B*), or rT3 (*C*). The stacking gel contained 5% acrylamide and the separatory gel contained 7.5% acrylamide. Gels were stained with Coomassie blue.

≈2-fold increase in cobalt heme-induced activity and a more modest increase in basal activity by 24 hr. These effects peaked at 48 hr and began to decline by 96 hr. A substantial decrease in cytochrome P-450 was evident at 96 hr in both groups of animals.

Effects of T3 on Microsomal Proteins. The polyacrylamide gel electrophoresis of microsomal proteins from thyroidectomized animals treated with T3 revealed a loss of major bands in the region of M_r ≈50,000–55,000 compared with the profiles from animals treated with either rT3 or diluent (Fig. 4). Though less dramatic, higher molecular weight protein species (M_r 60,000–70,000) also were decreased. In contrast, T3 treatment appeared to have enhanced other protein bands in the M_r 90,000–100,000 region. When intact rats were given T3 at a dose of 15 μg/100 of body weight for 4 days, there was a similar pattern of protein depletion in the M_r ≈50,000–55,000 region. SKF-525A, which complexes to cytochrome P-450 (28) and protects the heme moiety of the protein from degradation even in the presence of markedly induced levels of heme oxygenase (29), partially preserved these microsomal proteins from T3-induced depletion (data not shown). The cytochrome P-450 content in these microsomal preparations paralleled the electrophoretic results.

DISCUSSION

This report demonstrates that thyroid hormone can exert a significant regulatory effect on the rate of heme oxidation and on cytochrome P-450 content in the liver. T3 enhanced both basal and cobalt heme-induced heme oxygenase activity in a dose-dependent manner; this augmentation of the rate of heme oxidation was associated with a concurrent depletion of cytochrome P-450. At a dose of T3 known to restore thyroidectomized animals to the euthyroid state (26), both basal and cobalt heme-inducible heme oxygenase activities were restored to levels similar to those in sham-operated controls. These thyroid hormone effects were stereospecific, because they could be elicited by T3 but not by rT3, an analogue that is biologically inactive (30). The onset of the hormone effect on hepatic heme oxygenase after a single injection of T3 occurred within 24 hr and was maximal by 48 hr, with a substantial decrease in cytochrome P-450 content becoming evident by 96 hr.

Hormonal influences on hepatic cytochrome P-450 content and on oxidative chemical metabolism dependent on this heme protein have been described previously. When growth hor-

mone has been exogenously supplied to adult male rats, it has been shown to produce a decrease in cytochrome P-450 content and a concomitant decline in drug oxidation activity (31). A number of metabolites of natural steroid hormones also have been shown to induce this heme protein in avian liver (32). In macrophages involved in erythrophagocytosis, hydrocortisone has been reported to completely suppress heme oxygenase induction (33). Recently, we demonstrated that adrenalectomy results in an enhancement of basal and cobalt chloride-induced heme oxygenase activity and that hydrocortisone reverses this effect (34). In late pregnancy in the rat the chemical inducibility of δ-aminolevulinate synthase is greatly impaired, whereas the inducibility of heme oxygenase by cobalt chloride is not inhibited (35). Although insulin, glucagon, and epinephrine stimulated enzyme activity, thyroxine in massive doses (10 mg/100 g of body weight) was reported to be without effect on hepatic heme oxygenase when administered 7 and 5 hr prior to killing in rats (36). The absence of a thyroid hormone effect in the latter study may have resulted from the suboptimal time interval between hormone administration and the determination of the enzyme activity. However, the cytochrome P-450-depleting property of large amounts of thyroid hormone has been demonstrated previously in rat liver (13–16). In these studies animals were given either single doses or multiple injections of hormone and were killed 3–10 days after the initial treatment. A more recent report has described a dose-dependent decrease in this heme protein with as little as 5 μg/100 g of thyroxine daily for 7 days (11). Heme oxygenase activity was not measured in the latter study.

The findings of this study suggest that the thyroid hormone-mediated depletion of cytochrome P-450 content in liver may be the consequence, in part, of an enhanced rate of degradation of heme through the induction of heme oxygenase. However, the magnitude of the increase of basal heme oxygenase activity (≈2-fold) produced by the hormone may not fully account for the substantial decrease in the hepatic content of this hemeprotein (Fig. 2). Thus, thyroid hormone may act through an alternate mechanism as well, possibly by decreasing the amount of apocytochrome available for heme binding. A direct regulation of apoprotein has been suggested for the cytochrome P-450 depletion that results from iron–dextran administration (37). A similar level of regulation has been proposed for the increased hepatic cytochrome P-450 content after phenobarbital administration in rats (38). In the present study there appeared to be a concurrent loss of spectrally determined cytochrome P-450 (Fig. 2) and electrophoretically determined microsomal proteins migrating in the appropriate region (Fig. 4). Recently reported evidence suggests that thyroid hormone attenuates certain specific messenger RNA sequences while enhancing others in rat liver (26). Thus, it is possible that T3 might exert a regulatory influence on apocytochrome P-450 synthesis at the pretranslational level as well as by depletion of cellular heme through the induction of heme oxygenase.

The proximate molecular mechanism by which thyroid hormone regulates heme oxygenase in liver is not known. It is possible that the hormone acts as a direct inducer of the enzyme. The heme oxygenase response does share certain biochemical characteristics with previously identified actions of thyroid hormone in liver. For example, T3-mediated augmentation of sodium-dependent cellular respiration and the activities of Na^+,K^+-activated adenosine triphosphatase and glycerol-3-phosphate dehydrogenase in rat liver peak 48–96 hr after hormone treatment *in vivo* (39) and *in vitro* (40); *de novo* protein synthesis has been shown to be involved in the latter responses (41, 42). The maximal effect of T3 on both basal and cobalt heme-inducible heme oxygenase activity in this study occurred 48 hr after T3 treatment (Fig. 3) and this response was stereospecific with respect to T3 (Fig. 2), a characteristic of receptor-mediated hormone–cell interactions. The doses of thyroid hormone required to enhance heme oxygenase activity and deplete cytochrome P-450 (Fig. 1) also were similar to those that induce malic enzyme and glycerol-3-phosphate dehydrogenase in the rat liver *in vivo* (43). Alternatively, T3 enhancement of heme oxygenase may be mediated indirectly. Thyroid hormone exerts protean biological effects in the whole animal and the relationship of the hormone to the liver is complex because this organ serves both as a major site of hormone action as well as of hormone biotransformation and disposal. Thus, T3 regulation of heme oxygenase could involve secondary metabolic processes, such as those underlying the permissive actions of hormones in the chemical and steroid induction of hepatic δ-aminolevulinate synthase (19, 20). Studies that utilize primary cultures of liver cells growing in chemically defined medium may define this regulatory mechanism further.

The ability of T3 to alter significantly heme oxygenase activity and cytochrome P-450 content in liver extends the spectrum of natural hormones that now have been shown to influence key aspects of heme and heme protein metabolism and emphasizes the importance of the endocrine milieu of this organ in determining its adaptive responses to chemical exposures.

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