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Regulation of *CYP4A* Expression in Rat by Dehydroepiandrosterone and Thyroid Hormone

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SUMMARY

Dehydroepiandrosterone (DHEA) is a peroxisome proliferating agent when administered in pharmacological dosages, but it has not been shown to function through the peroxisome proliferator-activated receptor in cell-based assays. Because members of the thyroid hormone/vitamins A and D nuclear receptor subfamily, including PPAR, are known to modulate each other's function in gene expression by heterodimerization, we sought to establish whether DHEA and thyroid hormone interact to regulate several of the hepatic and renal enzymes associated with peroxisome proliferation, i.e., peroxisomal β-oxidation and microsomal NADPH:cytochrome P450 oxidoreductase and the cytochromes P450 4A. In rats administered exogenous T_3 to attain a hyperthyroid state, induction of the three isozymes of *CYP4A* (*4A1*, *4A2*, and *4A3*) by DHEA was suppressed >60–80% at the mRNA level, with induction of CYP4A2 mRNA being completely inhibited. Nuclear run-on transcription assays indicated that this inhibitory effect was regulated at the level of transcription. Induction of hepatic peroxisomal β-oxidation by DHEA or the peroxisome proliferator nafenopin was in large part unaffected by treatment of animals with T_3 under any condition tested. Microsomal NADPH:cytochrome P450 oxidoreductase activity was induced by either DHEA or T_3; cotreatment resulted in an additive induction. When animals were treated with a lower dose of exogenous T_3 that rendered the animals slightly hyperthyroid, only induction of hepatic CYP4A2 mRNA by DHEA or nafenopin was significantly inhibited (>80%) compared with euthyroid control animals. Animals that had been rendered hypothyroid through removal of the thyroid gland showed normal induction of *CYP4A* genes by DHEA in liver, suggesting that their induction by DHEA was not dependent on the presence of thyroid hormone. The administration of exogenous T_3 to thyroidectomized rats in the presence of DHEA potently suppressed hepatic induction of all three genes at the mRNA and protein level. In experiments with cultured rat hepatocytes, physiological concentrations of T_3 potently inhibited the induction of CYP4A2 mRNA levels by nafenopin but had little effect on induction of CYP4A1 or 4A3 mRNA. At higher T_3 concentrations, the induction of CYP4A1/4A3 mRNA and protein was also inhibited. These results suggest that T_3 modulates the expression of *CYP4A2* at the level of transcription in physiologically relevant concentrations but that hyperthyroid conditions are required to suppress expression of *CYP4A1/4A3* genes. In euthyroid rodent kidney, which only expresses *CYP4A2* under either basal or DHEA-induced conditions, near-physiological levels of T_3 caused potent suppression of peroxisome proliferator-dependent induction of *CYP4A2* mRNA levels by either DHEA or nafenopin. In thyroidectomized rats, basal expression of *CYP4A2* mRNA was decreased relative to euthyroid controls, but DHEA was as effective an inducer of this mRNA as it is in euthyroid rats. As seen in euthyroid rats, T_3 administration potently suppressed DHEA induction of *CYP4A2* mRNA levels under either basal or induced conditions. Although *CYP4A* expression was not derepressed in liver or kidneys of hypothyroid animals, our results indicated that the thyroid status of the animal did affect basal expression of *CYP4A2*, suggesting involvement of thyroid hormone or some other factor regulated by the thyroid gland on its constitutive expression.

In rat, three isozymes of the cytochrome P450 4A (*CYP4A*)[1] gene family have been identified and characterized; they are designated *CYP4A1*, *CYP4A2*, and *CYP4A3* (1–3). The *CYP4A* isozymes catalyze the ω- and ω-1 hydroxylation of fatty acids, including arachidonic acid and the ω-hydroxylation of prostaglandins in liver and kidney (4–6). These isozymes and their hydroxylated products may be important in hepatic lipid metabolism and detoxification of foreign chemicals, as well as in normal and pathological renal function. Induced expression of the *CYP4A* isozymes has been associated with peroxisome proliferation, a pathophysiological condition characterized by hepatomegaly, proliferation of peroxisomes, and increased peroxisomal β-oxidation of fatty acids (7–9). Peroxisome proliferation and increased microsomal and peroxisomal oxidation of fatty acids occurs in response to the administration to rodents of a diverse class of chemicals, including fatty acids, hypolipidemic agents, phthalate plasticizers, and phenoxyacid herbicides. Per-

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[1] The gene/isoform designations used for cytochrome P450 adhere to the systematic nomenclature recommended by Nelson *et al.* (1).

ABBREVIATIONS: ADIOL, 5-*ene*-androsten-3β,17β-diol; DHEA, dehydroepiandrosterone; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; PPAR, peroxisome proliferator-activated receptor; T_3, 3,3′,5-triiodothyronine; PO, oral; IP, intraperitoneal; CYP, cytochrome P450.

oxisome proliferation in rodent liver has also been correlated with certain nutritional states, i.e., high-fat diets (10), vitamin E-deficient diets (11), and cold adaption (12).

We have previously reported that the administration of supraphysiological dosages of the adrenal steroids DHEA and ADIOL to rats results in the induction of expression of all three *CYP4A* genes in liver and *CYP4A2* in kidney (13, 14). Other steroid hormones did not affect the expression of these *CYP4A* genes. In liver, all three *CYP4A* isozymes are normally expressed at very low basal levels in male Sprague-Dawley rats, and DHEA administration results in a 3–10-fold increase in the hepatic and renal levels of mRNA for the three *CYP4A* isozymes in a developmental and tissue-specific manner. Microsomal NADPH:cytochrome P450 oxidoreductase and peroxisomal catalase and β-oxidation are also induced by DHEA and ADIOL in rodent liver (14). In kidney, *CYP4A2* is expressed constitutively, and the mRNA levels for this isozyme are induced 2–3-fold on administration of DHEA; *CYP4A1* and *CYP4A3* are not expressed in kidney at significant levels constitutively or during peroxisome proliferation.

Our previous work demonstrated that induction of hepatic mRNAs of the *CYP4A* genes by DHEA occurs as a result of increased rates of transcription, comparable to the rate observed with induction by clofibrate, a potent peroxisome proliferator (14). Many peroxisome proliferators have been shown to increase the rate of transcription of genes induced in peroxisome proliferation by activation of the PPAR, a member of the steroid/thyroid hormone receptor superfamily (15–19). However, DHEA does not function as an inducing chemical in a variety of cell-based assays that use PPAR-expression plasmids and PPAR-responsive reporter genes (15, 20). In the present study, we sought to further characterize the regulation of the *CYP4A* isozymes by DHEA and nafenopin and the mechanism by which this adrenal steroid activates peroxisome proliferation.

DHEA induces the transcription of other genes involved in lipid metabolism, i.e., malic enzyme and *sn*-glycerol-3-phosphate dehydrogenase, both of which are transcriptionally regulated by thyroid hormone (21, 22). Interestingly, the regulation of these enzymes by DHEA is affected by the thyroid hormone status of the animal. Song *et al.* demonstrated that the induction of malic enzyme by DHEA required circulating thyroid hormone (22). Su and Lardy (21) did not observe an absolute requirement of T_3 for DHEA induction of malic enzyme or *sn*-glycerol-3-phosphate dehydrogenase but showed that DHEA induction of these enzymes was significantly decreased in hypothyroid animals. Thus, it was of interest to determine whether DHEA induction of the *CYP4A* isozymes and peroxisome proliferation also required thyroid hormone or was affected by the thyroid status of the animal.

Our findings demonstrate that the thyroid status of the animal affects DHEA regulation of the *CYP4A* isozymes but does so in an inhibitory fashion. Although DHEA induction did not absolutely require the presence of thyroid hormone, induction of CYP4A2 mRNA was considerably decreased in the livers of thyroidectomized animals, suggesting that thyroid hormone may in some part contribute to the action of DHEA. Furthermore, the normally high level of basal expression of *CYP4A2* in kidney was also inhibited by thyroid hormone in a dose-dependent manner. Recent studies have demonstrated the importance of *CYP4A*-catalyzed hydroxylation of arachidonic acid in renal hemodynamics; thus, thyroid hormone inhibition of *CYP4A2* expression may significantly influence normal renal function (23–25). Taken together, our results demonstrate that the *CYP4A* genes are regulated independently of other gene markers of peroxisome proliferation, such as microsomal NADPH:cytochrome P450 oxidoreductase and peroxisomal β-oxidation enzymes.

Experimental Procedures

Materials. DHEA and T_3 were purchased from the Sigma Chemical Co. (St. Louis, MO). Nafenopin was a gift from CIBA-GEIGY Co. (Ardsley, NY). Laboratory chow (AIN-76A) was obtained from ICN Biomedicals (Cleveland, OH); a separate portion of this chow was prepared to contain 0.45% (w/w) DHEA. Radiolabeled nucleotide triphosphates were obtained from DuPont-NEN Research Products (Boston, MA). The cDNAs for GAPDH, rabbit cytochrome P450 4A4, and rat NADPH:cytochrome P450 oxidoreductase cDNA (pOR7) were kindly provided by J. M. Blanchard [University des Sciences et Techniques du Languedoc, Montpellier, France (26)], E. F. Johnson [Scripps Institute and Clinic, La Jolla, CA (27)], and C. Kasper [McArdle Institute, University of Wisconsin, Madison, WI (28)], respectively. The *CYP4A* isozyme-specific oligonucleotides were synthesized according to the method of Sundseth and Waxman (29). The polyclonal anti-ω-hydroxylase immunoglobulin was graciously provided by Richard Okita (Washington State University, Pullman, WA). All other reagents were of high purity and were obtained from commercial sources.

Animal treatment. Male Sprague-Dawley rats (100 g) and sham-operated or thyroidectomized rats used for the hypothyroid studies were obtained from Harlan Sprague-Dawley (Indianapolis, IN). The sham-operated and thyroidectomized rats were obtained 1 week after surgery and ranged in weight from 130 to 150 g at the onset of treatment. They were maintained on 4% calcium lactate in their drinking water for the duration of treatment to prevent calcium depletion resulting from removal of the parathyroid gland during the thyroidectomy. DHEA (80 mg/kg body weight in corn oil) or nafenopin (40 mg/kg body weight) were administered by daily IP injections. Alternatively, DHEA was administered PO in a synthetic AIN-76A diet containing 0.45% (w/w) DHEA. T_3 (a near-physiological dose of 10 μg/100 g body weight or a hyperthyroid dose of 50 μg/100 g body weight) was administered daily by IP injection. The thyroid hormone stock solution was prepared by 1:10 dilution in saline of a 3.75 mg/ml solution in 0.1 N NaOH (22). The animals were anesthetized with CO_2 before being killed by decapitation.

Quantification of thyroid hormone levels. Free and total T_3 serum concentrations were determined in plasma by radioimmunoassay using the 1-FT3 MAGIC and T3 MAGIC kits, respectively (CIBA-Corning, Medfield, MA).

Preparation of subcellular fractions. Subcellular fractionation of liver tissue was performed by differential centrifugation according to the method of Remmer *et al.* (30). Livers perfused with saline were excised and either immediately frozen in liquid N_2 or used fresh for the preparation of supernatant or microsomal fractions that had been prepared by centrifugation at 5,000 × *g*. For peroxisomal palmitoyl-CoA oxidase activity measurements, supernatant fractions of liver homogenates (also prepared by 5,000 × *g* or 108,000 × *g* centrifugation) were prepared in 50 mM potassium phosphate buffer, pH 7.4, containing 0.25 M sucrose. For microsomal preparations, liver sections were pooled according to treatment groups and homogenized in 4 volumes (ml/g tissue) of 50 mM potassium phosphate buffer, pH 7.4, containing 0.25 M sucrose. The final microsomal pellet was resuspended in 0.5 volume (ml/g of original tissue) of a 50 mM potassium phosphate buffer, pH 7.4, containing 0.25 M sucrose and 1 mM EDTA. The various tissues and protein fractions were stored at −70°. Protein concentrations of liver micro-

somal fractions were determined using the Pierce BCA Protein Assay Reagent (Pierce, Rockford, IL) (31). Bovine serum albumin was used as a standard.

Enzyme assays. Microsomal NADPH:cytochrome P450 oxidoreductase activity was determined as described by Yasukochi and Masters (32). KCN-insensitive peroxisomal β-oxidation of fatty acids was determined with liver supernatants (prepared by 5000 × *g* centrifugation) by measuring NADH produced from the oxidation of the C3 hydroxyl group of palmitoyl-CoA catalyzed by 3-hydroxyacyl-CoA dehydrogenase according to the method of Lazarow (33).

Western immunoblot analysis of protein fractions. Microsomal proteins were electrophoretically separated on 10% sodium dodecyl sulfate-polyacrylamide gels according to the method of Laemmli (34) as described by Towbin *et al.* (35). Protein/immunoglobulin complex formation was detected with the use of enhanced chemiluminescent light emission of luminal oxidation with horseradish peroxidase using Amersham ECL Western blotting detection reagents (Amersham International, Arlington Heights, IL). The immune complexes observed on autoradiograms were quantified with the use of a Bio-Rad videodensitometer (model 620; La Jolla, CA).

Northern analysis of RNA fractions. Total mRNA was isolated as previously described by Prough *et al.* (14) according to the method of Chomczynski and Sacchi (36). The RNA was stored at −70° in ethanol. Total RNA was enriched for poly(A)$^+$ mRNA using the PolyATract mRNA isolation kit from Promega Corp. (Madison, WI) as described by Prough *et al.* (14). Total or poly(A)$^+$-enriched RNA was electrophoretically separated in a 1% agarose/15% formaldehyde gel, and the mRNA was transferred onto Zeta Probe nylon membranes (BioRad) through capillary action in 10× standard saline citrate. The RNA was immobilized onto the membrane through UV irradiation for 10 min on each side of the membrane. The cDNAs used as probes for Northern hybridization were radiolabeled using the Random Primed DNA labeling kit (Boehringer Mannheim, Indianapolis, IN), and the mRNA/DNA hybrids were formed according to the method optimized for Zeta Probe nylon membrane (Bio-Rad, Hercules, CA). The oligonucleotides specific for the individual *CYP4A* isozymes used for Northern hybridization were radiolabeled at their 5′ ends using a T4 polynucleotide kinase reaction. The hybridization complexes for specific mRNAs were measured with the use of the Bio-Rad videodensitometer. Kidney or liver tissue (~1 g) was pooled from three animals to prepare total RNA for analysis. Although the data presented represent a single preparation, each experiment was performed in duplicate or triplicate and the densitometer measurements varied no more than 15–20% between preparations.

***In vitro* transcriptional analysis.** To measure relative rates of transcription, we performed *in vitro* transcriptional analysis with isolated hepatic nuclei from treated animals as described previously (37). Male Sprague-Dawley rats were treated by IP injection of DHEA (80 mg/kg body weight in corn oil), T_3 (50 μg/100 g body weight), or both for 1 day. The animals were killed 24 hr after treatment, and livers were prepared for nuclei isolation as previously described (38). The specific plasmids were linearized and applied under vacuum to Schleicher and Schuell BA-85 nitrocelluose sheets using a Schleicher and Schuell slot-blot apparatus. Newly transcribed [^{32}P]-UTP-labeled RNA samples were hybridized to the plasmids, *CYP4A4*, GAPDH, and pBS KS− (the parent vector for the *CYP4A4* cDNA). The relative rates of transcription were determined by densitometry using a Bio-Rad model 620 videodensitometer and normalized to GAPDH as described previously by Prough *et al.* (14).

Primary hepatocyte cell culture. Hepatocytes were prepared from male adult Sprague-Dawley rats (200–250 g) by *in situ* liver collagenase perfusion (39, 40). Cell viability (≥80–85%) was determined by Trypan blue exclusion. Hepatocytes were suspended (1 × 10^6 cell/ml) in arginine-free Eagle's minimum essential medium supplemented with L-ornithine and insulin/transferrin/sodium selenite medium supplement. Cells (3.0 × 10^6/3.0 ml) were seeded onto 60 × 15-mm tissue culture dishes that had been precoated with Matrigel. Once plated, the cells were maintained at 37° in a humidified atmosphere of 95% air/5% CO_2. The media were changed after 2 hours and 24 hr of culture. At 24 hr, 40 μM nafenopin and/or T_3 (1 nM to 1 μM) were added with fresh media. Equivalent amounts (≤1.0% v/v) of solvent added to control cells were found to not significantly alter cell viability, enzyme activity, or total mRNA levels. At 72 hr, the media were removed from the dishes by aspiration, the cells were washed with Dulbecco's phosphate-buffered saline (2 × 1 ml), and cellular protein and mRNA were isolated as described by Xiao *et al.* (41). The proteins were stable for up to 6 months at −70° when analyzed with the use of Western immunoblot analyses. The mRNA was isolated and analyzed as described above.

Results

Effects of DHEA on circulating thyroid hormone levels. Experiments were designed to determine whether T_3 affected the basal, DHEA-induced, or nafenopin-induced expression of the P450 4A isozymes. The comparison with nafenopin was included to demonstrate that the responses to thyroid hormone were the same regardless of the peroxisome proliferator used. Male Sprague-Dawley rats (150 g) were administered DHEA PO, T_3 IP, or both daily for 7 days. Measurement of serum free T_3 and total T_3 levels of the animals on sacrifice confirmed whether the dosage of T_3 administered indeed rendered the animals hyperthyroid (Table 1). Administration of supraphysiological levels of T_3 IP (50 μg/100 g body weight) daily for 7 days resulted in a large increase in free T_3 present in serum (from 5.2 ± 0.6 to 42.0 ± 8.7 pg/ml); total T_3 also increased, from 1.7 ± 0.2 ng/ml in normal animals to 13.8 ± 2.7 ng/ml in T_3-treated animals. Administration of DHEA resulted in a ~30% decrease in free and total T_3 levels relative to control but did not significantly affect free or total T_3 when coadministered with T_3 daily. Administration of either DHEA or T_3 decreased the average daily weight gain of the animals by 27% and 67%, respectively (Table 1). These results are consistent with the thermogenic effects of these compounds that have been previously described (42). The animals that received both DHEA and T_3, however, not only failed to gain weight at the rate of the control animals but also displayed a 23% decrease in their initial weight observed at the onset of treatment. Hepatomegaly was observed in all animals (euthyroid, hyperthyroid, and hypothyroid) that were administered DHEA, with or without T_3, except for thyroidectomized rats that were treated with DHEA and T_3.

The dose of T_3 administered in the initial experiment was supraphysiological and toxic to the animals, as demonstrated by their drastic weight loss. Therefore, a lower, more physiological dosage of T_3 IP (10 μg/100 g body weight) was administered daily to euthyroid animals alone or with DHEA or nafenopin for 7 days. Measurement of free and total serum T_3 levels confirmed that the dosage administered was near-physiological (Table 1), i.e., levels of circulating T_3 were normal or lower at 24 hr after treatment. As was seen with high T_3 doses, DHEA feeding lowered both free and total T_3 levels, as did the peroxisome proliferator nafenopin. Administration of T_3 at a daily dosage of 10 μg/100 g body weight also decreased free and total T_3 levels relative to the levels observed in euthyroid control animals. The decrease in free and total T_3 levels observed with T_3 administration may reflect feedback inhibition to the hypothalamus and pituitary, signaling a decrease in T_4/T_3 production.

TABLE 1

Free and total T_3 serum levels of euthyroid and thyroidectomized rats administered DHEA and/or T_3

Treatment	Free T_3	T_3RIA	Average weight % gain/day	Liver/body weight ratio
	pg/ml	*ng/ml*	*g*	
Experiment 1: Euthyroid animals, supraphysiological dose, 50 μg T_3/100 g body weight				
Control	5.2 ± 0.6	1.7 ± 0.2	6.2	5.2
DHEA[a]	3.6 ± 0.5[b]	1.4 ± 0.1[c]	4.5	7.8
T_3	42.0 ± 8.7[b]	13.8 ± 2.7[b]	2.0	4.9
DHEA plus T_3	45.0 ± 12.4	11.4 ± 3.5	−5.7	7.2
Experiment 2: Euthyroid animals, near-physiological dose, 10 μg T_3/100 g body weight				
Control	7.1 ± 0.7	1.8 ± 0.2	7.6	5.8
DHEA	4.7 ± 0.6[c]	1.4 ± 0.2[c]	3.0	7.9
T_3	4.0 ± 0.8[c]	1.4 ± 0.2[c]	4.0	4.9
DHEA Plus T_3	5.6 ± 0.7	1.3 ± 0.1[c]	−2.0	8.1
Nafenopin[d]	3.4 ± 0.3[b]	1.1 ± 0.1[b]	2.0	8.4
Nafenopin plus T_3	4.2 ± 2.4[c]	1.3 ± 0.6[c]	3.0	9.2
Experiment 3: Hypothyroid animals, near-physiological dose, 10 μg T_3/100 g body weight				
Sham-operated animals				
Control	5.3 ± 0.9	1.6 ± 0.3	7.8	5.5
DHEA	3.4 ± 0.4	0.1 ± 0.1	0.2	6.5
Thyroidectomized animals				
Control	1.6 ± 0.7	0.7 ± 0.3	1.0	3.8
DHEA	0.8[e]	0.4[e]	−1.0	4.9
T_3	7.1 ± 1.9[c]	2.5 ± 0.6[c]	1.2	3.8
DHEA plus T_3	21.6 ± 4.7[b]	6.6 ± 2.0[b]	−7.4	3.5

[a] Animals received DHEA PO (0.45% DHEA in AIN-76A chow).
[b] Statistically different from control animals (four per group; $p < 0.005$).
[c] Statistically different from control animals (four per group; $p < 0.05$).
[d] Animals received nafenopin IP (40 mg/100 g body weight).
[e] Below the levels of detection of the assays used.

In a third experiment, either sham-operated (euthyroid) or thyroidectomized (hypothyroid) male Sprague-Dawley rats were administered T_3 IP (10 μg/100 g body weight) daily for 6 days, which induces mild hyperthyroidism. The sham-operated euthyroid and thyroidectomized hypothyroid rats were either treated or not treated with DHEA PO for 7 days. Thyroidectomy itself significantly decreased the levels of free and total serum T_3 to levels just barely over the limits of detection (Table 1). The low levels of T_3 may reflect incomplete surgical removal of the thyroid gland or residual hormone in the circulation. Administration of DHEA to thyroidectomized animals decreased free and total serum T_3 levels below the limits of detection. Administration of T_3 (10 μg/100 g body weight) resulted in mild hyperthyroidism relative to euthyroid controls. Coadministration of DHEA and T_3 to the thyroidectomized animals significantly increased free and total serum T_3 levels nearly 3-fold over the levels measured in animals receiving T_3 alone. This result is consistent with a report by Lardy *et al.* that demonstrated synergism of DHEA on T_3 action in thyroidectomized animals that had been administered T_3 (42).

T_3 and DHEA both induce hepatic NADPH:cytochrome P450 oxidoreductase. NADPH-cytochrome P450 oxidoreductase has been shown to be induced by all peroxisome proliferators studied to date (7, 13). The rate of reduction of cytochrome *c* catalyzed by NADPH:cytochrome P450 oxidoreductase was measured in hepatic microsomal fractions prepared from animals treated with DHEA PO, supraphysiological doses of T_3 IP (50 μg/100 g body weight), or both. Both DHEA and T_3 induced the activity of this enzyme, by 1.8- and 1.7-fold, respectively, consistent with previous reports (15, 43). Coadministration of DHEA and T_3 resulted in an additive induction in enzyme activity (3.3-fold); these values correlated well with the increases observed in oxidoreductase protein content with the use of Western analysis and in mRNA levels with the use of Northern analysis (Fig. 1).

T_3 does not affect DHEA-induced peroxisome proliferation. The effects of T_3 and DHEA on peroxisomal β-oxidation of fatty acids were determined by measuring the activity of palmitoyl-CoA oxidase, the rate-limiting enzyme in

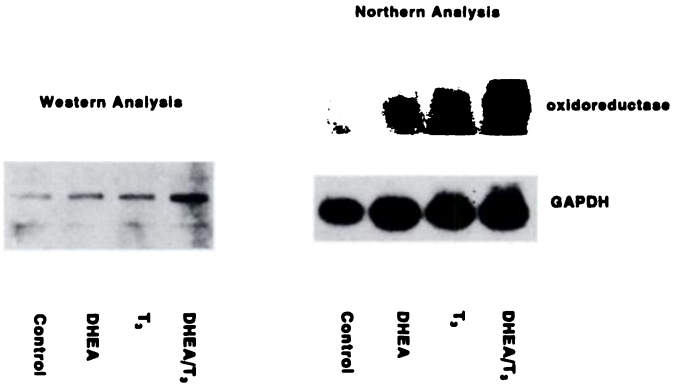

Fig. 1. Western immunoblot and Northern analysis of hepatic NADPH:cytochrome P450 oxidoreductase from rats treated with DHEA and/or high doses of T_3. Male Sprague-Dawley rats were treated with DHEA PO (0.45% DHEA in AIN-76A chow), T_3 IP (50 μg/100 g body weight), or both daily for 7 days. For the Western analysis, liver microsomal fractions (20 μg of protein) were separated electrophoretically on 10% sodium dodecyl sulfate-polyacrylamide gels, transferred to nitrocellulose membranes, and incubated with an anti-NADPH:cytochrome P450 oxidoreductase globulin. The immunoreactive complexes were visualized by enhanced chemiluminescent techniques. For the Northern analysis, poly(A)$^+$-enriched mRNA was electrophoretically separated on 1% agarose/formaldehyde gels, transferred to nylon membranes, and hybridized with a ^{32}P-labeled cDNA specific for NADPH:cytochrome P450 oxidoreductase. The hybridization complexes of the Northern analysis were visualized by autoradiography and quantified by densitometry; the optical density values were control, 1.0; DHEA, 1.9; T_3, 1.4, and DHEA/T_3, 4.6.

the pathway. A previous report demonstrated that administration of T_3 had a slight inhibitory effect on hepatic palmitoyl-CoA oxidase activity in rats (44). As seen in Fig. 2, the activity of peroxisomal palmitoyl-CoA oxidase was not significantly affected by administration of T_3 alone (10 μg/100 g body weight), but the activity of palmitoyl-CoA oxidase activity was induced >7-fold by administration of either DHEA or nafenopin. Furthermore, thyroid hormone had no effect on the level of induction of hepatic palmitoyl-CoA oxidase activity by DHEA at either 10 (Fig. 2) or by 50 μg T_3/100 g body weight IP (data not shown). A small inhibitory effect of T_3 was observed with nafenopin (Fig. 2). These results indicate that T_3 does not exert as significant an inhibitory effect on induction of peroxisomal β-oxidation and peroxisome proliferation as it does on induction of CYP4A2.

T_3 inhibits induction of the hepatic *CYP4A* mRNAs by DHEA. Because T_3 state apparently did not affect peroxisomal β-oxidation, initial experiments were designed to determine whether T_3 affected either the basal or DHEA-induced expression of the P450 4A isozymes. In this study, Sprague-Dawley rats (150 g) were administered DHEA PO, T_3 IP (50 μg/100 g body weight, which causes a hyperthyroid condition), or both daily for 7 days. Northern analysis was performed to determine whether T_3 affected DHEA-induced expression of the *CYP4A* genes through a pretranslational mechanism. Oligonucleotide probes specific for mRNA of the individual *CYP4A* genes were used to identify which of the isozymes were affected (29). Administration of high doses of T_3 (50 μg daily/100 g body weight for 7 days) inhibited the induction of the *CYP4A1* and *CYP4A3* mRNA levels by DHEA ~60–70% (Fig. 3). However, the induction of *CYP4A2* mRNA by DHEA was completely inhibited by T_3.

A lower, more physiological dose of T_3 (10 μg/100 g body weight) was administered to euthyroid animals alone or with DHEA for 7 days. To determine whether T_3 would inhibit the induction of the P450 4A isozymes by other peroxisome proliferators, T_3 was also coadministered with nafenopin, a potent peroxisome proliferator. Northern analysis of hepatic total mRNA hybridized to the isozyme-specific oligonucleotide probes is presented in Fig. 4. The lower dosage of T_3 inhibited induction of *CYP4A2* mRNA by DHEA by 80%. Induction of *CYP4A2* by nafenopin was also inhibited by T_3 administration (by 65%). The lower dosage of T_3, however, apparently did not inhibit the induction of *CYP4A1* and *CYP4A3* mRNA by DHEA but caused, at best, a slight increase, 1.5- and 1.2-fold, respectively. The induction of *CYP4A1* and *CYP4A3* mRNA by nafenopin was likewise only slightly inhibited by T_3 (Fig. 4). These results indicate that T_3 inhibits the induction of the *CYP4A2* isozyme by peroxisome proliferators in a physiologically relevant, concentration-dependent manner. The inhibition of DHEA induction of *CYP4A1* and *CYP4A3* by thyroid hormone, however, may be more complex, requiring supraphysiological concentrations.

T_3 inhibition of induction of hepatic *CYP4A* isozymes by DHEA is mediated at the level of transcription. DHEA induction of the *CYP4A* mRNAs was observed to increase the rate of transcription of the *CYP4A* genes (14). *In vitro* transcription run-on assays were performed on isolated hepatic nuclei from untreated animals and on animals treated with DHEA PO, T_3 IP (50 μg/100 g body weight), or both to determine whether the inhibition of *CYP4A* mRNA levels by T_3 occurred at the level of transcription. The relative rates of transcription of *CYP4A* for the different treatments were determined by densitometry and normalized to GAPDH. The rate of transcription of hepatic *CYP4A* 24 hr after a single dose of DHEA was increased 2.2-fold above control (Fig. 5). This value is lower than the 11-fold induction of *CYP4A* transcription observed when DHEA was administered for 2 days (15) and may reflect a need for bioaccumulation of DHEA or an active intermediate of DHEA action as a peroxisome proliferator or synthesis of a limiting cellular factor. T_3 decreased the induction in *CYP4A* transcription by DHEA ≥40%. This value is consistent with the partial inhibition of *CYP4A* mRNA levels observed with the use of Northern analysis when T_3 was coadministered with DHEA. However, the relative rate measured may represent a composite rate of all three *CYP4A* isozymes due to the high degree of sequence similarity of the rat *CYP4A* structural genes to the rabbit CYP4A4 gene (2, 3, 27). *CYP4A1* and *CYP4A3* mRNA expression was only partially inhibited by T_3, whereas *CYP4A2* expression was completely inhibited. Therefore, only partial inhibition of the rate of

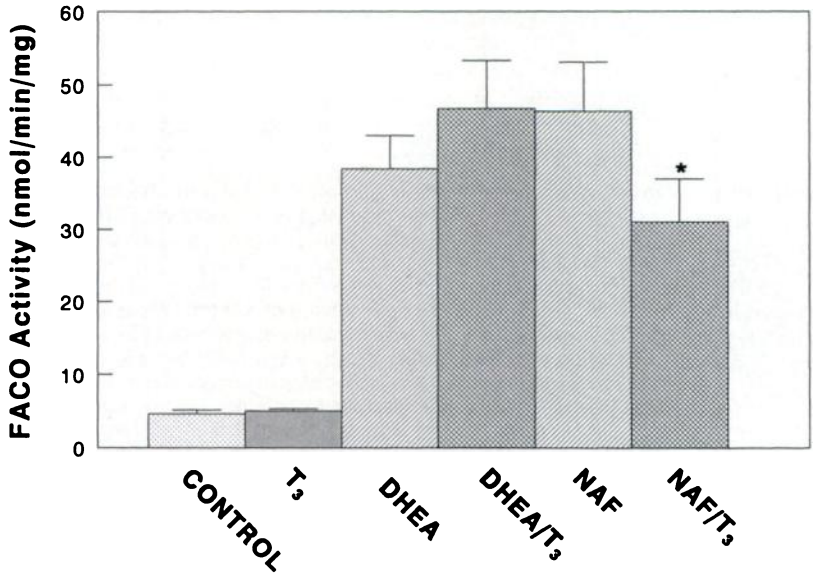

Fig. 2. Hepatic peroxisomal β-oxidation in rats treated with DHEA, nafenopin, and/or near-physiological doses of T_3. Male Sprague-Dawley rats were treated with DHEA PO (0.45% DHEA in AIN-76A chow), T_3 IP (10 μg daily/100 g body weight), or both daily for 7 days. Peroxisomal β-oxidation of palmitoyl-CoA was assayed spectrophotometrically in hepatic supernatant fractions that had been centrifuged at 3000 × *g*. The activity is reported as nmol NADH produced/min/mg protein (average ± standard deviation, four rats). *, Significantly different from nafenopin treatment ($p \leq 0.015$).

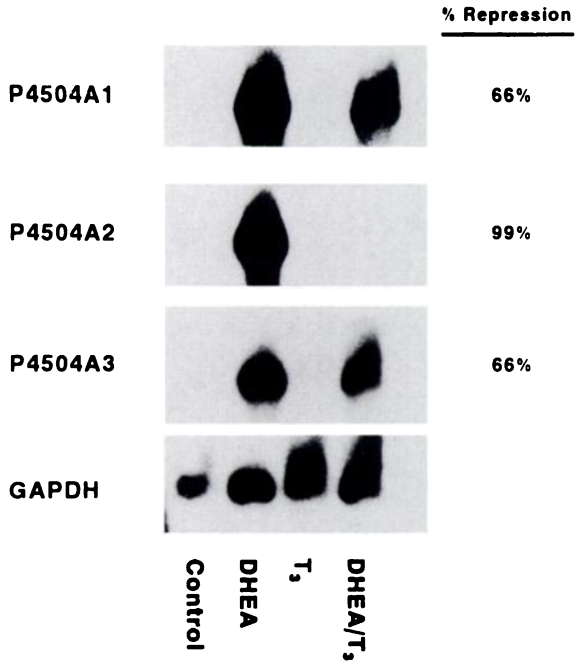

Fig. 3. Northern analysis of hepatic poly(A)$^+$-enriched mRNA from male rats treated with DHEA and/or high doses of T_3. Male Sprague-Dawley rats were administered DHEA PO (0.45% DHEA in AIN-76A), T_3 IP (50 μg/100 g body weight), or both daily for 7 days. Poly(A)$^+$-enriched mRNA was electrophoretically separated on 1% agarose/formaldehyde gels, transferred to nylon membranes, and hybridized to ^{32}P-labeled oligonucleotides specific to the individual P450 4A isozymes or the cDNA for GAPDH. The hybridization complexes were visualized with autoradiography, measured with densitometry, and normalized to GAPDH mRNA levels. Values represent the percentage repression obtained by dividing the normalized optical densities of DHEA/T_3 groups by the value for the DHEA groups.

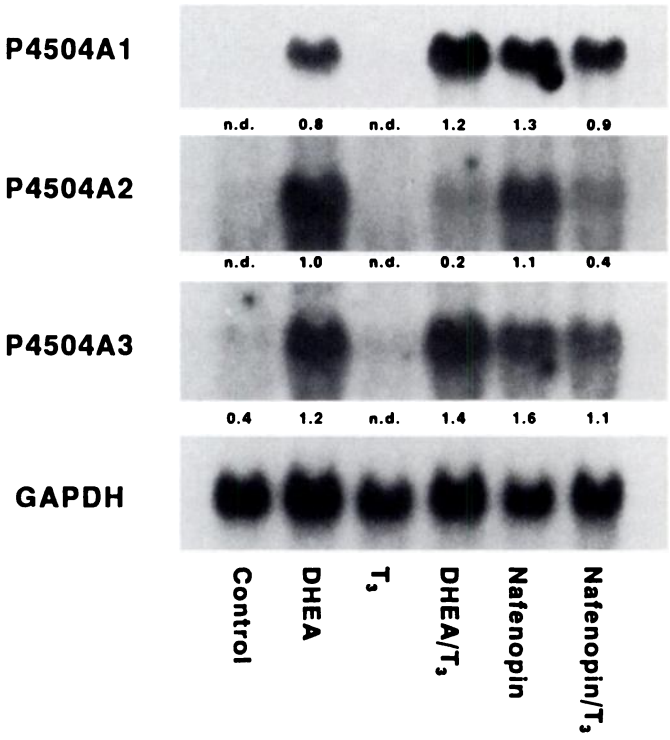

Fig. 4. Northern analysis of hepatic mRNA from male rats treated with DHEA and/or a near-physiological dose of T_3. Male Sprague-Dawley rats were administered DHEA PO (0.45% DHEA in AIN-76A chow), T_3 IP (10 μg/100 g body weight), nafenopin IP (50 mg/kg body weight), or various combinations of those compounds daily for 7 days. Total RNA was analyzed as described in legend to Fig. 3 using a ^{32}P-labeled oligonucleotide specific for the individual P450 4A isozymes or the cDNA for GAPDH. Values are the actual optical densities of the hybridization complexes obtained from the autoradiograms, normalized to GAPDH mRNA levels.

DHEA-induced transcription by T_3 would be expected if the rate measured is a composite of all three isozymes. The results of the *in vitro* transcriptional analysis are consistent with a transcriptional mechanism for inhibition of DHEA-induced expression of the P450 4A family isozymes by T_3.

Induction of the hepatic *CYP4A* mRNA levels by DHEA is affected by thyroid hormone state. The expression of genes that are negatively regulated by thyroid hormone is believed to be derepressed when animals are rendered hypothyroid by surgical thyroidectomy. Because DHEA induction is altered in a hyperthyroid state, the absence of thyroid hormone may also affect its ability to induce the *CYP4A* isozymes. Therefore, it was of interest to examine both basal and DHEA-induced expression of the *CYP4A* isozymes in animals of differing thyroid hormone status. In this experiment, sham-operated (euthyroid) or thyroidectomized (hypothyroid) male Sprague-Dawley rats were administered DHEA PO, T_3 IP daily (10 μg/100 g body weight, which induces hyperthyroidism), or both for 7 days.

Northern analysis of hepatic poly(A)$^+$-enriched mRNA hybridized to the isozyme-specific oligonucleotide probes is presented in Fig. 6. There was no apparent derepression of expression of any of the *CYP4A* isozymes in the hypothyroid control animals. The absolute level of induced hepatic *CYP4A2* mRNA by DHEA was significantly diminished (~90%) in the hypothyroid relative to the euthyroid animals. This effect was not observed with *CYP4A1* and *CYP4A3* mRNA levels in liver; in fact, their induction was slightly elevated in the hypothyroid animals. In striking contrast, DHEA induction of mRNA for all three isozymes was inhibited 50–75% when T_3 was administered to thyroidectomized animals. These results indicated that the low levels of *CYP4A* isozyme basally expressed in liver are not the result of an active repression by T_3, as indicated by the absence of derepression of expression of these hemoproteins in the hypothyroid animals. Furthermore, DHEA induction of the *CYP4A1* and *CYP4A3* isozymes was observed in both euthyroid and hypothyroid animals, indicating that DHEA induction of these proteins is not dependent on normal circulating levels of thyroid hormone. However, thyroid hormone status apparently affects DHEA induction of hepatic *CYP4A2* mRNA, as the relative level of induced *CYP4A* mRNA in hypothyroid animals was significantly diminished relative to the level of induction observed in euthyroid animals. The transition from a hypothyroid to a mild hyperthyroid state on the administration of T_3 (10 μg/100 g body weight) decreased the extent of induction of all three *CYP4A* isozymes by DHEA, which is consistent with previous observations in intact hyperthyroid animals (Fig. 4).

T_3 inhibits induction of P4504A isozymes by DHEA. Western immunoblot analysis of hepatic microsomal protein with an anti-rat ω-hydroxylase immunoglobulin is presented in Fig. 7. There were no observed differences in the induction (~5-fold) of the immunoreactive P450 proteins by DHEA with regard to euthyroid or hypothyroid hormone state. DHEA in-

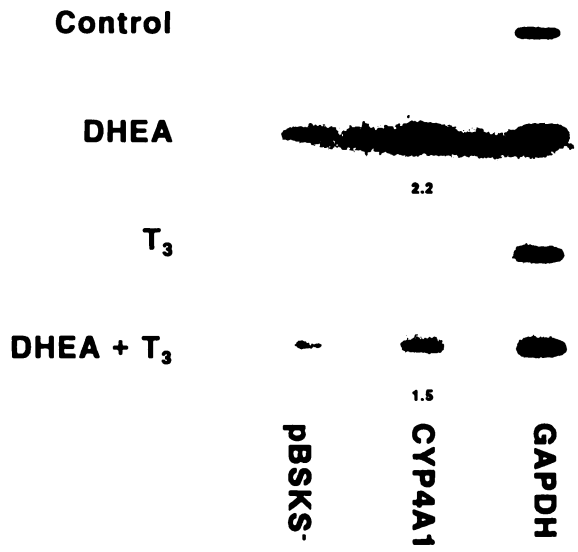

Fig. 5. *In vitro* transcriptional analysis of hepatic nuclei from male rats treated with DHEA and/or high doses of T_3. Male Sprague-Dawley rats (140–160 g) were treated with DHEA IP (80 mg/kg body weight), T_3 IP (50 μg/100 g body weight), or both. Nuclei were prepared from fresh liver tissue and frozen at −70°. *In vitro* transcription was initiated in the presence of ^{32}P-labeled UTP. The newly transcribed RNA was isolated; hybridized to pBS KS−, p4A4 (cDNA from rabbit *CYP4A4*), and pNUC5 (cDNA from GAPDH) immobilized onto nitrocellulose membranes; and visualized with the use of autoradiography. The relative rates of transcription are presented as the optical densities of the relevant bands corresponding to the hybridization complexes normalized to the levels of GAPDH mRNA. Data represent the fold induction by DHEA (2.2-fold) or by DHEA/T_3 (1.5-fold).

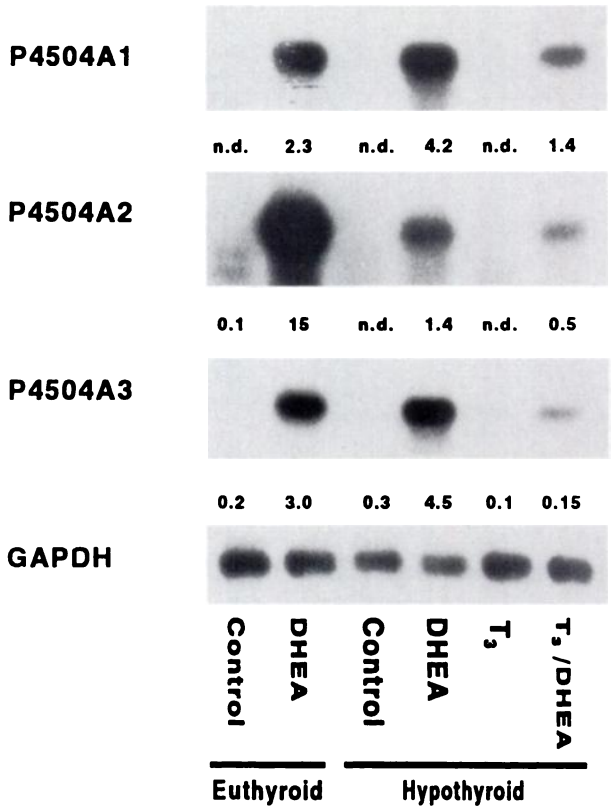

Fig. 6. Northern analysis of hepatic poly(A)$^+$-enriched mRNA from euthyroid and thyroidectomized male rats treated with DHEA and/or near-physiological doses of T_3. Sham-operated euthyroid, thyroidectomized hypothyroid, and thyroidectomized hyperthyroid (administered 10 μg/100 g body weight of T_3 IP) rats were treated with DHEA PO (0.45% DHEA in AIN-76A chow) daily for 6 days. Poly(A)$^+$-enriched mRNA was analyzed as described in legend to Fig. 3 using a ^{32}P-labeled oligonucleotide specific for the individual P450 4A isozymes or the cDNA for GAPDH. Values are the actual optical densities of the hybridization complexes obtained from the autoradiograms, normalized to GAPDH mRNA levels.

duction of the proteins was inhibited by >75% when T_3 (50 μg/100 g body weight) was coadministered to the thyroidectomized animals (Fig. 7). The results presented in Fig. 7 indicate that the decrease in protein content of the *CYP4A* family that was observed resulted from a decrease in the steady state mRNA levels for the different isozymes in response to T_3 administration (Fig. 3). A second experiment with thyroidectomized rats displayed a 90% inhibition (data not shown). Nearly identical results (~50–60% inhibition) were observed in euthyroid animals that were treated with DHEA and 50 μg T_3/100 g body weight (data not shown). These results also demonstrated that T_3 administration significantly decreased the content of an immunochemically related protein not induced by DHEA (14) that was constitutively expressed in the livers of control animals. The identity of this protein is not known. It may be another isozyme of the *CYP4A* family, such as *CYP4A5*, which is constitutively expressed in liver and kidneys of rabbits but is not induced by clofibrate (6). No corresponding orthologue has been identified in rat liver. Administration of T_3 inhibited the DHEA-induced expression of the *CYP4A* family and possibly its basal expression, which is in contrast to the apparent lack of effect of T_3 on peroxisomal β-oxidation and the additive induction of NADPH:cytochrome P450 oxidoreductase in animals fed DHEA.

T_3 inhibits basal and peroxisome proliferator-induced *CYP4A2* expression in kidney. Cytochrome P4504 A2 is expressed constitutively in the kidneys of mature male rats, and both DHEA and ADIOL are effective inducers of renal *CYP4A2* (14). We examined the effect of thyroid hormone on the basal and DHEA-induced expression of *CYP4A2* in kidney. Northern analyses of total RNA isolated from kidneys of animals treated with DHEA PO, nafenopin IP, near-physiological doses of T_3 IP (10 μg/100 g body weight), or combination of both peroxisome proliferator and hormone are presented in Fig. 8. Expression of *CYP4A2* in kidney is induced ~3-fold by DHEA administration over the normal basal level of expression. Thyroid hormone treatment inhibits both the basal and the DHEA-induced expression of *CYP4A2* by ~40–60%. Induction of renal *CYP4A2* mRNA by the peroxisome proliferator nafenopin was also inhibited >60% by administration of the lower dosage of thyroid hormone (Fig. 8). Administration of higher doses of T_3 (50 μg/100 g body weight) more effectively inhibited both the basal and induced expression of renal *CYP4A2* mRNA by ~80–90% (data not shown).

Thyroid status differentially regulates renal *CYP4A2* expression. Basal and DHEA-induced mRNA expression of renal *CYP4A2* was evaluated in animals of differing thyroid hormone states, i.e., when sham-operated (euthyroid) or thyroidectomized (hypothyroid) rats were administered a slightly greater than replacement dose of T_3 (10 μg IP/100 g body weight) and fed DHEA. Northern analysis of total mRNA from kidney hybridized to a *CYP4A2*-specific oligonucleotide probe is presented in Fig. 9. In the

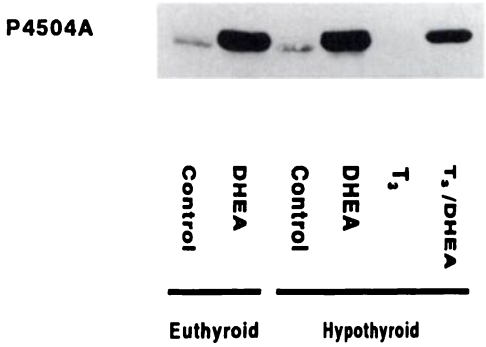

Fig. 7. Western immunoblot analysis of hepatic microsomal cytochrome P450 4A from euthyroid and hypothyroid male rats treated with DHEA and/or near-physiological doses of T_3. Sham-operated euthyroid, thyroidectomized hypothyroid, and thyroidectomized hyperthyroid (administered 10 μg/100 g body weight of T_3 IP) rats were treated with DHEA PO (0.45% DHEA in AIN-76A chow) daily for 6 days. Liver microsomal fractions (20 μg protein) were analyzed using an anti-*CYP4A* globulin as described in legend to Fig. 1. The relative optical densities of relevant bands on the autoradiogram corresponding to the immune complexes were measured; these optical density values were euthyroid control, 1.0; euthyroid DHEA, 5.1; thyroidectomy control, 1.0; thyroidectomy DHEA, 4.9; thyroidectomy T_3, 0.3, and thyroidectomy, DHEA/T_3, 1.3.

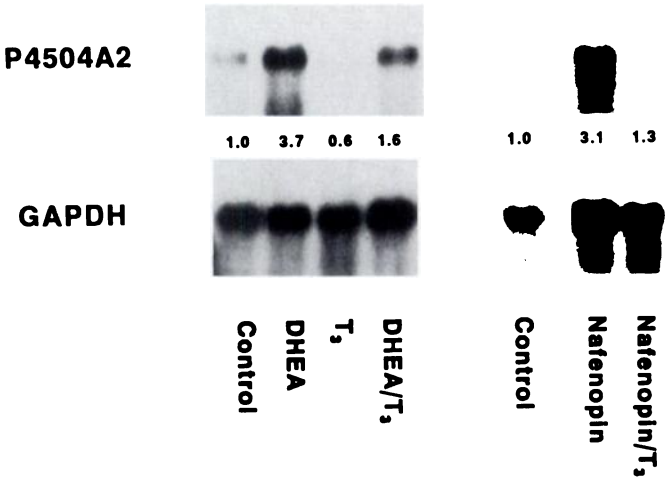

Fig. 8. Northern analysis of renal mRNA of male rats treated with DHEA, nafenopin and/or near-physiological doses of T_3. Male Sprague-Dawley rats were treated with DHEA PO (0.45% DHEA in AIN-76A chow), nafenopin IP (40 mg/kg body weight), T_3 IP (10 μg/100 g body weight), or a combination of the compounds daily for 7 days. Renal total RNA was analyzed as described in legend to Fig. 4 using a ^{32}P-labeled oligonucleotide specific for P4504 A2 or the cDNA for GAPDH. Values are the actual optical densities of the hybridization complexes obtained from the autoradiograms, normalized to GAPDH mRNA levels.

euthyroid, sham-operated animals, DHEA induced renal *CYP4A2* expression by 1.8-fold, which is comparable to the 2-fold induction observed in intact animals (14). DHEA caused nearly identical levels of induction in thyroidectomized rats. These data indicate that the induction of renal *CYP4A2* expression by DHEA is not dependent on the presence of T_3 and that thyroidectomized rat kidney is as responsive to DHEA treatment as those of euthyroid rats. However, when the thyroidectomized animals were administered DHEA along with a dosage of T_3 that would render them hyperthyroid, the level of induction by DHEA was strikingly diminished (>80%).

The basal level of *CYP4A2* mRNA expression in thyroidectomized hypothyroid animals was considerably lower than the basal level observed in the sham-operated euthyroid animals. These findings indicate that thyroid hormone regulation of renal *CYP4A2* expression is complex and may involve other hormonal factors. The decreased basal expression in the hypothyroid animals suggests that normal function of the thyroid gland is probably required for basal expression observed in the euthyroid animals, yet hyperthyroid levels of T_3 inhibited renal *CYP4A2* expression. Due to the lower levels of basal expression, the fold induction of *CYP4A2* mRNA was greater in thyroidectomized relative to sham-operated, euthyroid rats (6.5- versus 1.6-fold).

T_3 regulates induction of *CYP4A* by nafenopin in cultured rat hepatocytes. To establish whether T_3 acts directly on the hepatocyte, we used primary cultures of rat hepatocytes to determine the effect of graded concentrations of T_3 on the expression of *CYP4A* mRNA (41). We established that nafenopin, a peroxisome proliferating agent, was capable of inducing P450 4A protein in adult rat hepatocytes at the level of protein and mRNA (data not shown). We noted that concentrations ≤50 μM nafenopin caused increasing levels of P450 4A protein and mRNA for the various isozymes of *CYP4A* by 10–20-fold at 72 hr (data not shown); concentrations higher than this were toxic to the cells, as measured with Trypan blue exclusion and lactate dehydrogenase leakage. DHEA was not an effective inducer of *CYP4A* isozymes in this culture system (45).[2] We subsequently tested the effect of increasing concentrations of T_3 on nafenopin-dependent induction of *CYP4A* mRNAs (Fig. 10). T_3 specifically suppressed the induction of mRNA specific for *CYP4A2* by nafenopin at concentrations as low as 1×10^{-9} M, whereas concentrations as high as 1 μM T_3 had no statistically significant effect on induction of *CYP4A1* and *CYP4A3* mRNA. These results are nearly identical to the effect of T_3 on induction of *CYP4A2 in vivo* at the lower T_3 dose, demonstrating that T_3 apparently acts directly at the level of the hepatocyte in regulating *CYP4A2* expression and not by altering other hormonal factors associated with the thyroid or parathyroid gland.

Discussion

DHEA has been described as thyromimetic (21, 42, 46). That is, the administration of pharmacological levels of DHEA to rodents elicits effects on intermediary metabolism that are similar to those of supraphysiological levels of thyroid hormone. The administration of either DHEA or T_3 to rats significantly decreased body weight, fatty acid synthesis, and mitochondrial respiration and increased total body oxygen consumption. These effects have been suggested to be mediated through the coordinate regulation of several enzymes involved in lipid homeostasis by DHEA and T_3. The regulation of malic enzyme by DHEA required the presence of T_3 *in vivo* (21). We have sought to determine whether the *CYP4A* isoenzymes were similarly regulated by DHEA and T_3 and whether DHEA induction of these isozymes required T_3. In the present study, we report that DHEA and T_3 do not coordinately regulate the expression of the *CYP4A* family of

[2] X.-D. Lei and R. A. Prough, unpublished observations.

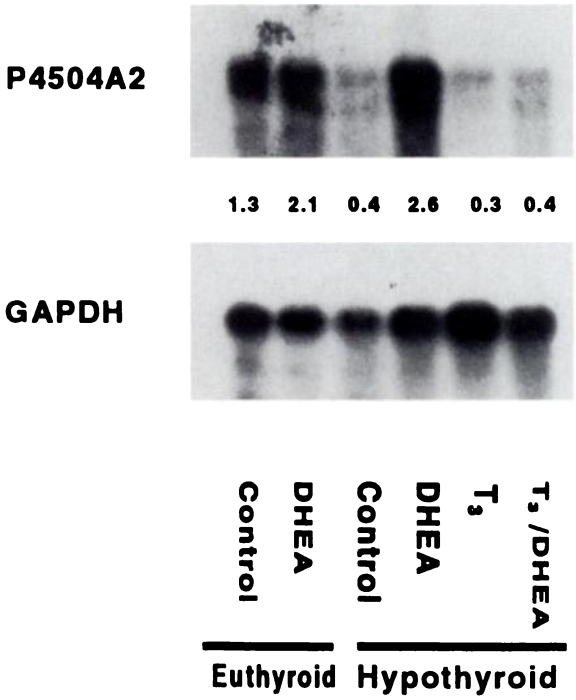

Fig. 9. Northern analysis of renal mRNA from euthyroid, hypothyroid, and hyperthyroid male rats treated with DHEA and/or near-physiological doses of T_3. Sham-operated euthyroid, thyroidectomized hypothyroid, and thyroidectomized hyperthyroid (administered 10 μg/100 g body weight of T_3 IP) rats were treated with DHEA PO (0.45% DHEA in AIN-76A chow) daily for 6 days. Total RNA isolated from kidney was analyzed as described in legend to Fig. 3 using a ^{32}P-labeled oligonucleotide specific for P4504 A2 or the cDNA for GAPDH. Values are the actual optical densities of the hybridization complexes obtained from the autoradiograms normalized to GAPDH mRNA levels.

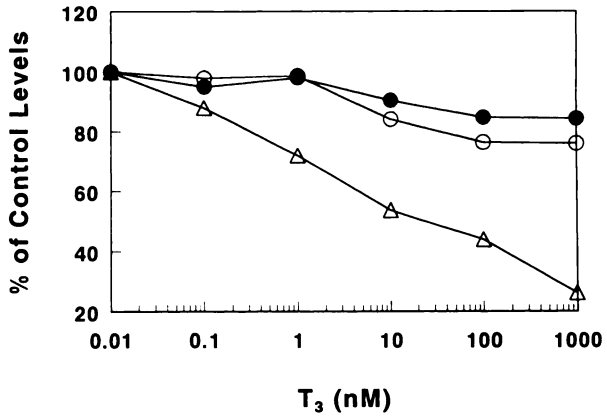

Fig. 10. Effect of thyroid hormone on nafenopin induction of *CYP4A* mRNAs in cultured rat hepatocytes. After being plated onto Matrigel-coated dishes, the cells were cultured for 24 hr. Nafenopin (40 μM) was then added to the media with increasing concentrations of T_3. After 72 hr, the cells were collected, and the mRNA was isolated. Total mRNA was analyzed as described in legend to Fig. 4 using a ^{32}P-labeled oligonucleotide specific for various *CYP4A* genes or the cDNA for GAPDH. Values presented are the actual optical densities of the hybridization complexes obtained from the autoradiograms normalized to GAPDH mRNA levels. The data shown represent a single experiment; however, this experiment was performed three times with nearly identical results obtained at 100 nM T_3 (±15%). ○, *CYP4A1*; △, *CYP4A2*; ●, *CYP4A3*.

isozymes in rat but that supraphysiological levels of T_3 oppose induction of the *CYP4A* family, strongly inhibiting the expression of these isozymes when administered with DHEA or nafenopin. Furthermore, we established that DHEA induction of the *CYP4A* isozymes did not appear to absolutely require the presence of circulating thyroid hormone.

In animals rendered hyperthyroid by the administration of a supraphysiological dosage of T_3 (50 μg/100 g body weight), the induction of hepatic *CYP4A* protein content and mRNA levels by DHEA was significantly inhibited. Western analysis of hepatic microsomal fractions prepared from animals administered DHEA, T_3, or both revealed that thyroid hormone significantly inhibited the DHEA induction of *CYP4A* protein content by >50%. When thyroid hormone was administered alone, an immunologically related, constitutively expressed protein, which was not induced by DHEA (14), was also suppressed. The identity of this protein is not known; however, it may be a rat orthologue to *CYP4A5*. Northern analysis revealed that the steady state mRNA levels for hepatic *CYP4A1* and *CYP4A3* were also decreased 60–70% when T_3 was coadministered with DHEA, whereas the induction of *CYP4A2* by DHEA was completely inhibited. Thyroid hormone inhibition of DHEA-induced expression of *CYP4A2* was also observed when a more physiological dose of T_3 (10 μg/100 g body weight) was administered. In addition, thyroid hormone inhibited induction of the *CYP4A2* isozymes by the potent peroxisome proliferator nafenopin.

We also assessed DHEA induction of hepatic *CYP4A* isozymes in animals rendered hypothyroid by surgical thyroidectomy. Because thyroid hormone inhibited peroxisome proliferator-induced expression of *CYP4A* isozymes, it was possible that thyroid hormone, under physiological conditions, exerted a repressive effect on *CYP4A* expression and that removal of the thyroid gland might result in a derepression of *CYP4A* expression. Negative regulation of other cytochromes P450 by thyroid hormone has been demonstrated (47, 48). However, *CYP4A* isozyme expression was not derepressed in hypothyroid animals, although the thyroid status of the animal was shown to have an effect on DHEA inducibility of *CYP4A2* (negative) and *CYP4A1* *CYP4A3* (slight positive). Induction of the *CYP4A* isozymes by DHEA was not absolutely dependent on the presence of thyroid hormone. This is in contrast to the observation by Song *et al.* (22) that DHEA required thyroid hormone to induce malic enzyme. DHEA induction of *CYP4A2* in the livers of hypothyroid rats was significantly lower than that observed in euthyroid animals, suggesting involvement of thyroid hormone or some other factor regulated by the gland in DHEA-regulated *CYP4A2* expression. DHEA induction of *CYP4A1* and *CYP4A3* by DHEA was not diminished in the hypothyroid animals.

Although thyroid hormone inhibited peroxisome proliferator-induced expression of the *CYP4A* isozymes, thyroid hormone did not significantly inhibit the induction of hepatic peroxisome proliferation by either DHEA or nafenopin. The administration of T_3 at near-physiological dosages had little or no effect on DHEA- or nafenopin-induced palmitoyl-CoA oxidase activity (data not shown). Induction of NADPH:cytochrome P450 oxidoreductase by T_3 plus DHEA was additive. In addition, hepatomegaly, a characteristic of peroxisome proliferation, was observed in all euthyroid and hyperthyroid animals treated with the peroxisome proliferators DHEA and nafenopin, except hypothyroid animals treated with both

DHEA and T_3. Thus, thyroid hormone inhibition of peroxisome proliferator-induced expression of the *CYP4A* isozymes apparently is not seen for other enzymes induced by peroxisome proliferators.

Several studies have reported that induction of *CYP4A* ω-hydroxylase activity temporally precedes the induction of peroxisomal enzymes and subsequent peroxisome proliferation (49). It has been proposed that the ω-hydroxylated fatty acids or other products formed may serve as substrates for the peroxisomal β-oxidation pathway and that peroxisome proliferation may be an adaptive response to increased levels of these substrates. Our findings of significantly decreased *CYP4A* expression in the presence of normal levels of peroxisome proliferation do not support this model. Of course, *CYP4A2* and *CYP4A3* expression was only partially inhibited by T_3, and thus there may be sufficient ω-hydroxylase activity to induce peroxisome proliferation, if it is required. Although T_3 has been reported to be a weak peroxisome proliferator when administered to rats at a dose of 20 μg/100 g body weight (44), we did not observe appreciable hepatomegaly or induction in peroxisomal β-oxidation of fatty acids when thyroid hormone was administered at a dose of either 10 or 50 μg/100 g body weight in our study.

Of particular interest was the effect of thyroid hormone on *CYP4A2* expression in kidney. Administration of both supraphysiological and physiological dosages of thyroid hormone whether alone or with DHEA resulted in significant diminution in the normally high levels of basal *CYP4A2* expression in kidney. Renal P450-dependent ω- and ω-1-hydroxylation of arachidonic acid has been demonstrated in rabbit and rat and is likely catalyzed by members of the *CYP4A* family (5, 6). The regulation of *CYP4A* isozymes in kidney by DHEA and T_3 and the subsequent effects on arachidonic acid metabolism may, therefore, have a significant effect on normal and pathological functions. Induction of *CYP4A2* expression in kidney has been observed in spontaneously hypertensive rats, suggesting that the function of this enzyme may be involved in hypertension (50, 51). Clearly, further investigation of the regulation of *CYP4A2* by DHEA and thyroid hormone in kidney and the potential effects on renal function and disease is warranted.

In a previous report, we demonstrated that induction of *CYP4A1* expression by DHEA occurred at the level of transcription (14), as has been shown for another peroxisome proliferator, clofibrate. To establish whether thyroid hormone inhibited DHEA induction of *CYP4A* expression through inhibition of transcription, we performed *in vivo* transcription run-on analysis of nuclei isolated from animals treated with DHEA, T_3, or both. Coadministration of thyroid hormone (50 μg/100 g body weight) with DHEA resulted in a 40% decrease in the rate of transcription of the *CYP4A1* gene relative to the level observed when DHEA was administered alone. The level of inhibition of transcriptional activity by T_3 correlated strongly with the decrease observed in the steady state mRNA levels of hepatic *CYP4A1* of rats receiving both DHEA and thyroid hormone. The addition of thyroid hormone to primary cultured rat hepatocytes resulted in a concentration-dependent decrease in nafenopin-induced *CYP4A2* expression with a significant 60% decrease in *CYP4A2* mRNA levels when thyroid hormone was coadministered at 10^{-8} M. Thyroid hormone did not significantly decrease *CYP4A1/CYP4A3* mRNA levels in cultured rat hepatocytes, which is similar to the result observed *in vivo*. The differences in the degree of inhibition of the different *CYP4A* isozymes by thyroid hormone suggest the possibility of different mechanisms of inhibition at low and high concentrations relative to peroxisomal β-oxidation and microsomal NADPH: cytochrome P450 oxidoreductase.

Several mechanisms for thyroid hormone-mediated repression of gene transcription has been described. Carr *et al.* (52, 53) demonstrated thyroid hormone-dependent repression of transcription of the gene for the β subunit of thyroid hormone-stimulating hormone via thyroid receptor binding to a negative thyroid hormone-responsive element located within the 5′-upstream region of the gene. Our findings, at least for *CYP4A2*, suggest that the inhibition of expression may be the result of a direct receptor-mediated event, as proposed by Carr *et al.* (53) and Piedrafita *et al.* (54), as thyroid hormone inhibits basal expression of *CYP4A2* in kidney as well as peroxisome proliferator-induced expression in a dose-dependent manner. These results along with our finding that thyroid hormone inhibited *CYP4A2* expression *in vitro* in primary cultured hepatocytes at endocrinologically and physiologically significant concentrations support direct receptor-mediated regulation of this gene. The inhibition of peroxisome proliferator-induced expression of *CYP4A1* and *CYP4A3* at high T_3 levels, however, may be mediated through a different mechanism because partial inhibition of *CYP4A1* and *CYP4A3* peroxisome proliferator-induced expression occurred only when supraphysiological doses of T_3 were administered. However, the observation that acyl-CoA oxidase expression was largely unaltered by T_3 would rule out a common mechanism involving sequestration of retinoid X receptor by liganded thyroid hormone receptor (55). Our future studies will address possible mechanisms for thyroid hormone-mediated inhibition of *CYP4A* isozyme expression, including identification of regions within the genes responsible for inhibition.

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