Hormonal regulation of cholesterol 7α-hydroxylase specific activity, mRNA levels, and transcriptional activity in vivo in the rat

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Abstract In primary cultures of rat hepatocytes, transcription of the cholesterol 7α-hydroxylase gene is induced synergistically by glucocorticoid and thyroid hormones. The objective of the present study was to evaluate the role of endogenous glucocorticoid and thyroid hormones in the maintenance of cholesterol 7α-hydroxylase gene expression in vivo. Male Sprague-Dawley rats underwent adrenalectomy (A), thyroidectomy (T), adrenalectomy + thyroidectomy (A + T), hypophysectomy (H), or sham surgery (paired controls). Ten days post surgery, livers were harvested and cholesterol 7α-hydroxylase specific activity, steady-state mRNA levels, and transcriptional activity were determined. Serum corticosterone levels were <2% of paired controls in A, A + T, and H rats. Free thyroxine index was <32% of paired controls in rats with T and H. When compared to sham-operated controls, A + T and H led to decreases in cholesterol 7α-hydroxylase specific activities of 44 ± 8% and 57 ± 3%, respectively ($P < 0.03$ and < 0.05). Similar changes were observed in cholesterol 7α-hydroxylase steady-state mRNA levels, which decreased by 43 ± 10% ($P < 0.001$) and 56 ± 19% ($P < 0.05$), respectively. Cholesterol 7α-hydroxylase transcriptional activity in A + T and H rats decreased by 34 ± 11% ($P < 0.01$) and 61 ± 4% ($P < 0.001$), respectively. The observed decreases were greater after H than after A + T, suggesting the possibility that another pituitary hormone plays a role in regulation of cholesterol 7α-hydroxylase. Thyroidectomy alone led to a decrease in cholesterol 7α-hydroxylase specific activity of 37 ± 7% ($P < 0.05$) and a trend toward decreased steady-state mRNA levels (21 ± 12%; P = ns). Adrenalectomy did not significantly decrease cholesterol 7α-hydroxylase specific activity or mRNA levels. Neither thyroidectomy nor adrenalectomy alone affected transcriptional activity. We conclude that under physiologic circumstances, full expression of the cholesterol 7α-hydroxylase gene requires synergistic action of glucocorticoids and thyroid hormone.—**Pandak, W. M., D. M. Heuman, K. Redford, R. T. Stravitz, J. Y. L. Chiang, P. B. Hylemon, and Z. R. Vlahcevic.** Hormonal regulation of cholesterol 7α-hydroxylase specific activity, mRNA levels, and transcriptional activity in vivo in the rat. *J. Lipid Res.* 1997. **38:** 2483–2491.

Supplementary key words cholesterol 7α-hydroxylase • gene • regulation • liver • cholesterol • glucocorticoid • thyroid

Microsomal cholesterol 7α-hydroxylase (EC 1.14.13.17), a member of the cytochrome P-450 (CYP7A) gene superfamily, catalyzes the initial and rate-determining step in the classic or neutral bile acid biosynthetic pathway. Until recently this pathway was thought to be the major route for elimination of cholesterol from the body (1, 2). Recent in vitro (3, 4) and in vivo studies (5) have shown that an alternative "acidic" pathway of bile acid synthesis, which is initiated by the mitochondrial cytochrome P-450 enzyme sterol 27-hydroxylase, is also probably a significant contributor to total bile acid synthesis. A clearer understanding of the relative importance of each of these pathways and how they are regulated is necessary if we are to fully understand hepatic cholesterol homeostasis.

Numerous in vitro and in vivo studies have shown that cholesterol 7α-hydroxylase transcriptional activity is down-regulated by hydrophobic bile acids (6–10) and up-regulated by cholesterol (11, 12). It has also been recently proposed that regulation of cholesterol 7α-hydroxylase by hydrophobic bile acids is mediated by protein kinase C (13, 14). Similarly, sterol 27-hydroxylase, the initial enzyme in the alternative pathway, is down-regulated by hydrophobic bile acids (15).

Other effectors and molecular mechanisms responsi-

Abbreviations: A, adrenalectomy; T, thyroidectomy; A + T or T + A, adrenalectomy + thyroidectomy; H, hypophysectomy.

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ble for maintenance of the basal level of cholesterol 7α-hydroxylase transcription in the liver have not been well defined. In primary rat hepatocytes, glucocorticoids (16), glucocorticoid plus thyroxine (17), and thyroxine (18) were described as stimulators of cholesterol 7α-hydroxylase. In contrast, insulin (19) and glucagon (17, 20) were described as repressors. In primary cultures of rat hepatocytes, cholesterol 7α-hydroxylase specific activity, mRNA, and transcriptional activity were not spontaneously expressed. Co-administration of glucocorticoids and thyroxine increased steady-state mRNA levels and transcriptional activity to the levels exceeding those observed in whole liver homogenates (17).

With the exception of the study of Ness et al. (18) prior studies on the molecular basis of hormonal regulation of cholesterol 7α-hydroxylase have been carried out only in primary rat hepatocytes. Results obtained in primary rat hepatocytes depend to a great extent on the culturing conditions, leading often to controversial results (16, 17, 21).

The present study represents the first attempt to define in an in vivo model the role of thyroxine and glucocorticoids on the regulation of cholesterol 7α-hydroxylase in rats after selective ablation of endocrine organs (adrenal, thyroid, pituitary). The data of the present study show that glucocorticoids and thyroid hormone synergistically contribute to up-regulation of basal expression of the cholesterol 7α-hydroxylase gene in vivo.

MATERIALS AND METHODS

Materials

All chemicals were of the highest grade available. [4-^{14}C]cholesterol (59.4 mCi/mmol), DL-[^{3}H]3-hydroxymethyl-3-glutaryl coenzyme A (57.6 mCi/mmol), and DL-[^{3}H]mevalonate (30 Ci/mmol) were obtained from New England Nuclear (Boston, MA). DL-Mevalonolactone, dithiothreitol, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, 3-hydroxy-3-methylglutaryl coenzyme A, NADP^{+}, NADPH, proteinase K, and ribonuclease A were obtained from Sigma Chemical Company (St. Louis, MO). Silica gel chromatography sheets were obtained from Fisher Scientific (Springfield, NJ). The nick translation kit was obtained from Bethesda Research Laboratories (Gaithersburg, MD). Guanidine thiocyanate was purchased from Fluka (Ronkonkoma, NY) and deoxyribonuclease I was obtained from Worthington. Sephadex G-50 (fine grade DNA) was purchased from Pharmacia LKB Biotechnology, Inc. (Piscataway, NJ).

Experimental design

Adrenalectomized (A), thyroidectomized (T), adrenalectomized and thyroidectomized (A + T or T + A), and hypophysectomized (H) male Sprague-Dawley rats (250–350 g) were purchased from Charles River, Cambridge, MA. Rats were housed in metabolic cages under controlled lighting conditions on a natural dark–light cycle (0600–1800 h light phase). Paired controls, age- and weight-matched and sham-operated, were used in all experiments. A and A + T rats had free access to NaCl (0.9%)-supplemented drinking water. Hypophysectomized rat drinking water was supplemented with 10% dextrose. After 10 days of paired feeding, rats were subjected to brief methoxyflurane anesthesia. Blood was collected after decapitation (6), and livers were harvested for further analysis.

HMG-CoA-reductase specific activity, cholesterol 7α-hydroxylase specific activity, and microsomal cholesterol

Microsomes were prepared as previously described (6). Microsomal protein was determined by the method of Bradford (22). Microsomal HMG-CoA reductase specific activity was assayed by the method of Whitehead et al. (23). The specific activity of cholesterol 7α-hydroxylase was determined in microsomes using reversed phase high performance liquid chromatography as previously described (24). Microsomal free cholesterol was quantified after digitonin precipitation by the method of Abell et al. (25).

Determination of steady-state mRNA levels for sterol 27-hydroxylase and cholesterol 7α-hydroxylase

Methods for isolation of RNA and determination of steady-state mRNA levels by Northern blotting have been described previously (6).

Briefly, pieces of liver (approximately 1 g each) were homogenized in a solution of 4 M guanidine thiocyanate, 10 mmol/L Tris (pH 7.4), 7% β-mercaptoethanol and 2% sarcosyl and then passed through a 23-gauge needle. After heating for 5 min to 65°C and centrifugation (3,000 rpm for 10 min), the supernatant was underlayered with 5.7 M cesium chloride and 10 mmol/L EDTA (pH 7.4) and centrifuged at 100,000 *g* for 16 h. The pellet was washed with 95% ethanol, suspended in distilled water, and stored at −70°C. RNA was then size-fractionated by means of electrophoresis, transferred to nylon membrane filters, and then covalently bound with membranes with UV crosslinker (Spectrolinker XL-1000 UV Crosslinker). Membranes were then hybridized with an α-^{32}P-labeled *EcoRI-AccI* digested fragment of pBSK7α6, containing a cDNA for cholesterol 7α-hydroxylase. The same membranes were subsequently washed and rehybridized to a radiolabeled cDNA encoding rat

albumin (pRSA57), used as an internal loading control (6). The absorbencies for hybridization to rat cholesterol 7α-hydroxylase and albumin were determined by laser densitometry for each condition. The ratios of the resulting indices were compared to paired controls.

Determination of transcriptional activity

Isolation of nuclei and nuclear run-on studies were carried out as previously described (6). In brief, nascent mRNAs of rat cholesterol 7α-hydroxylase, albumin, and cyclophilin were elongated in vitro in the presence of [^{32}P]GTP (nuclear run-on assay) (6, 26). Completed mRNAs were hybridized to their respective cDNAs vacuum-blotted to nitrocellulose membranes. The ratio of the intensity of cholesterol 7α-hydroxylase to albumin and cyclophilin after autoradiography was calculated for each membrane. Both albumin and cyclophilin were used as internal controls to insure there were no effects of hormonal manipulation on either control. Data are expressed as % of paired untreated controls (mean ± SE).

Serum determinations

Serum albumin, aspartate and alanine aminotransferase, bilirubin, alkaline phosphatase, triglyceride, cholesterol, and thyroid function studies were performed by the clinical laboratory at the McGuire Veterans Affairs Medical Center. Serum corticosterone levels were determined by Hazleton Washington Laboratories (Vienna, VA).

Statistical analysis

Statistical differences between groups were tested by means of student's *t*-tests for unequal samples, using the SAS statistical program (SAS Institute, Cary, NC).

RESULTS

Effects of surgical endocrine organ ablation on serum hormone concentrations and weight gain

As shown in **Table 1,** thyroidectomy (T), adrenalectomy plus thyroidectomy (A + T), and hypophysectomy (H) resulted in a 40–50% decrease in T3, and 68–73% decrease in T4. Surgical endocrine organ ablation decreased serum corticosterone levels by 97% after hypophysectomy and by >98% after adrenalectomy. Serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin, and total bilirubin values were not altered by surgical endocrine organ ablation (data not shown). The effects of endocrine organ ablation on animal weight gain over the duration of the study are illustrated in **Table 2.** Weight gain after thyroidectomy, A + T, and hypophysectomy was 36 ± 9% ($P < 0.001$), 67 ± 5% ($P < 0.001$), and 22 ± 10% ($P < 0.001$), respectively, of the weight gain by sham-operated paired controls over the course of the study. The weight of rats having undergone adrenalectomy alone was not significantly different from their paired controls.

TABLE 2. Effect of adrenalectomy (A), thyroidectomy (T), thyroidectomy plus adrenalectomy (T + A), and hypophysectomy (H) on weight gain over study duration as compared to sham-operated paired controls

	n	% of Control
A	8	92 ± 15
T	7	36 ± 9[a]
T + A	8	67 ± 5[a]
H	4	22 ± 10[a]

Data are expressed as mean ± SE.
[a] $P < 0.001$.

HMG-CoA reductase and cholesterol 7α-hydroxylase specific activities in endocrine organ-ablated rats

The effects of surgical endocrine organ ablation on HMG-CoA reductase and cholesterol 7α-hydroxylase specific activities (10 days after surgery) are shown in **Fig. 1** and **Fig. 2,** respectively. A mild decrease of HMG-CoA reductase specific activity after adrenalectomy (A), thyroidectomy (T), and A + T as compared to sham-operated paired controls was observed, but was not statistically significant. Hypophysectomy, however, was associated with a significant decrease in the activity of

TABLE 1. Effect of adrenalectomy, thyroidectomy, thyroidectomy plus adrenalectomy (T + A), and hypophysectomy on serum hormone levels as compared to sham-operated paired controls

	Adrenalectomy		Thyroidectomy		T + A		Hypophysectomy	
	%	n	%	n	%	n	%	n
Corticosterone	1.4	2			1	1	3	2
T_3	112 ± 15	8	51 ± 21[a]	7	53 ± 12[a]	6	60 ± 16	4
T_4	113 ± 8	8	32 ± 4[a]	7	28 ± 2[a]	8	27 ± 5[a]	4
Free T_4 index	96 ± 5	7	26 ± 4[a]	8	13 ± 3[a]	3	27 ± 10[a]	3

Data are expressed as percent of paired controls (mean ± SE).
[a] $P < 0.05$.

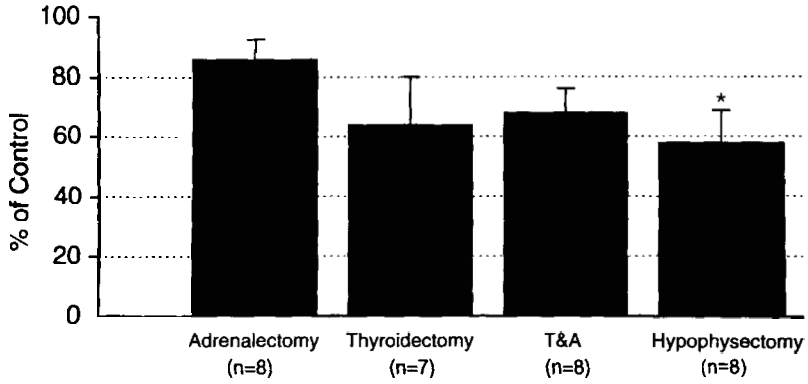

Fig. 1. Effects of surgical endocrine organ ablation on the specific activity of HMG-CoA reductase. The specific activity of HMG-CoA reductase was determined 10 days after adrenalectomy, thyroidectomy, A + T, and hypophysectomy, and is expressed as percent of sham-operated, paired controls (mean ± SE); $*P < 0.05$.

HMG-CoA reductase (↓42 ± 8%; $P < 0.05$). Cholesterol 7α-hydroxylase specific activity, however, was significantly decreased after thyroidectomy (↓37 ± 7%; $P < 0.005$), A + T (↓44 ± 8%; $P < 0.003$), and hypophysectomy (↓57 ± 3%; $P < 0.05$). Adrenalectomy alone decreased cholesterol 7α-hydroxylase specific activity modestly, but not significantly (↓25 ± 6%; $P < 0.06$).

HMG-CoA reductase and cholesterol 7α-hydroxylase mRNA levels and transcriptional activity in endocrine organ-ablated rats

Cholesterol 7α-hydroxylase mRNA levels showed statistically significant decrease after A + T (↓43 ± 10%; $P < 0.001$) or hypophysectomy (↓56 ± 19%; $P < 0.05$) as compared to sham-operated controls (**Fig. 3**). Decrease of cholesterol 7α-hydroxylase mRNA levels after adrenalectomy (↓23 ± 10%) and thyroidectomy (↓21 ± 12%) were not statistically significant.

A + T and hypophysectomy decreased cholesterol 7α-hydroxylase transcriptional activities (34 ± 11% and 61 ± 4%), respectively. These decreases were statistically highly significant ($P < 0.001$) and similar in magnitude to those observed with cholesterol 7α-hydroxylase specific activity and steady-state mRNA levels (**Fig. 4**). In contrast, neither adrenalectomy nor thyroidectomy alone significantly altered cholesterol 7α-hydroxylase transcriptional activity in hepatocellular nuclei as compared to sham-operated, paired controls. These experimental manipulations were specific for cholesterol 7α-hydroxylase, as the transcriptional activity of cyclophilin and albumin were not affected (**Fig. 5**).

Effect of surgical endocrine organ ablation on hepatic microsomal cholesterol, serum cholesterol, and serum triglycerides

Serum triglyceride and cholesterol levels in endocrine organ-ablated rats were not significantly different from sham-operated paired controls (data not shown). All experimental manipulations, however, increased hepatic microsomal free cholesterol (**Fig. 6**): adrenalectomy (↑26 ± 3%; $P < 0.01$); thyroidectomy (↑34 ± 5%; $P < 0.02$); A + T (↑20 ± 7%; P = ns); and, hypophysectomy (↑67 ± 10%; $P < 0.05$).

DISCUSSION

In vitro studies in primary rat hepatocytes have shown that expression of cholesterol 7α-hydroxylase is dependent upon the addition of glucocorticoids (16) or thyroxine plus glucocorticoids (17) to the culture

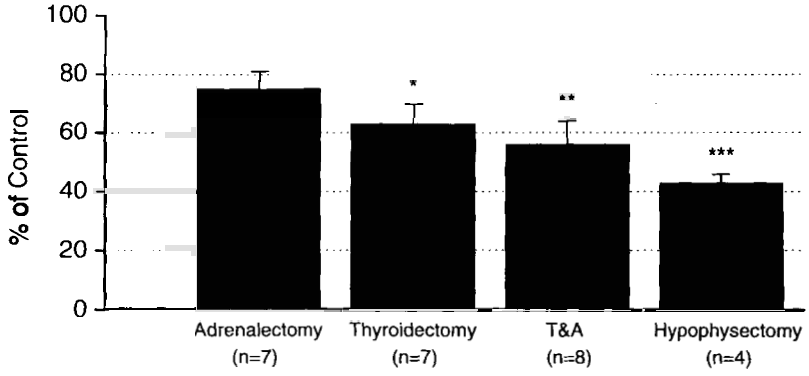

Fig. 2. Effect of surgical endocrine organ ablation on the specific activity of cholesterol 7α-hydroxylase. The specific activity of cholesterol 7α-hydroxylase was determined 10 days after surgical hormonal manipulation and is expressed as percent of sham-operated, paired controls (mean ± SE); $*P < 0.005$; $**P < 0.003$; $***P < 0.05$.

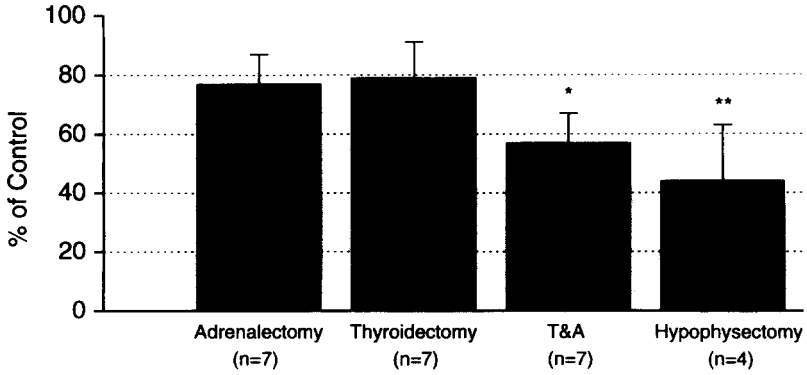

Fig. 3. Effect of surgical endocrine organ ablation on cholesterol 7α-hydroxylase mRNA levels. Steady-state mRNA levels of cholesterol 7α-hydroxylase 10 days after endocrine organ ablation are compared to levels in sham-operated, paired controls and controlled for variation of RNA loading as described in Materials and Methods. Data expressed as % of paired controls (mean ± SE); $*P < 0.001$; $**P < 0.05$.

medium. In contrast, sterol 27-hydroxylase, which is also down-regulated by hydrophobic bile acids, is fully expressed after addition of glucocorticoid alone (27). Furthermore, cholesterol 7α-hydroxylase and sterol 27-hydroxylase are subject to diurnal variation, suggesting a similar regulation by glucocorticoids (15). The lack of certainty about the synergistic action of glucocorticoids and thyroxine on cholesterol 7α-hydroxylase necessitated confirmation in an in vivo experimental model.

The present in vivo study in the rat demonstrates that under physiologic conditions thyroid hormone and glucocorticosteroids contribute to maintenance of basal expression of cholesterol 7α-hydroxylase. Only hypophysectomy or combined adrenalectomy plus thyroidectomy were found to consistently decrease cholesterol 7α-hydroxylase mRNA levels and transcriptional activity. The effects of isolated adrenalectomy or thyroidectomy on cholesterol 7α-hydroxylase specific activity mRNA levels and transcriptional activity were small and not statistically significant. Hypophysectomy resulted in a greater decrease in specific activities, mRNA levels, and transcriptional activity than adrenalectomy plus thyroidectomy, suggesting that other yet unidentified pituitary hormone(s) may also sustain cholesterol 7α-hydroxylase expression in vivo (28, 29). Previous studies have suggested that the ability of growth hormone to modulate cholesterol metabolism may occur as a function of its ability to stimulate bile acid synthesis via up-regulation of cholesterol 7α-hydroxylase activity (28). The finding that thyroidectomy decreased cholesterol 7α-hydroxylase specific activity without significantly reducing mRNA or transcriptional activity suggests that thyroid hormone may be involved in post translational regulation of the enzyme. Similar results were obtained in HepG2 cells that were transiently transfected with rat cholesterol 7α-hydroxylase promoter/luciferase reporter constructs (30).

The present observations support our previous findings in primary cultures of rat hepatocytes, in which cholesterol 7α-hydroxylase activity, mRNA, and transcriptional activity were not spontaneously expressed without the concomitant inclusion of thyroxine and dexamethasone to the culture medium (17). In these latter studies the addition of either dexamethasone or thyroxine alone increased cholesterol 7α-hydroxylase steady-state mRNA levels and transcriptional activity only slightly, while the addition of both hormones synergistically increased mRNA and transcriptional activity over 11-fold, i.e., to levels far above those found in whole liver in rats fed cholestyramine. Two other studies add additional support for our findings. Ness et al.

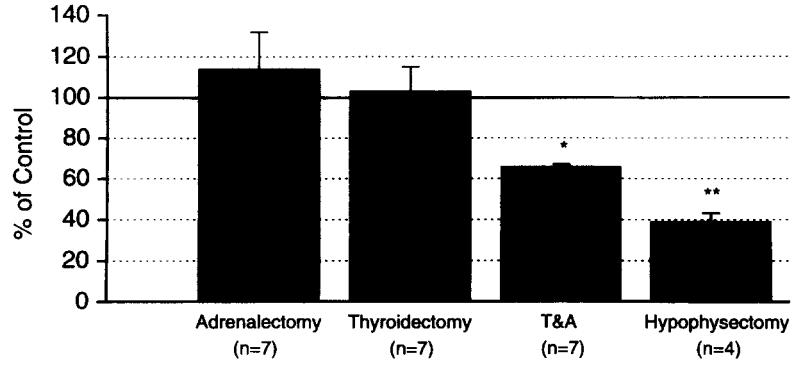

Fig. 4. Effect of adrenalectomy, thyroidectomy, thyroidectomy plus adrenalectomy, and hypophysectomy on cholesterol 7α-hydroxylase transcriptional activity. Nascent mRNAs of cholesterol 7α-hydroxylase and both rat albumin and cyclophilin were elongated in vitro in the presence of [^{32}P]GTP (nuclear run-on assay; see Methods). Completed mRNAs were then hybridized to their respective cDNAs and blotted to nitrocellulose membranes. The ratio of the intensity of cholesterol 7α-hydroxylase to cyclophilin and albumin after autoradiography was calculated by laser densitometry for each membrane. Data are expressed as % of sham-operated paired controls (mean ± SE); $*P < 0.01$; $**P < 0.001$.

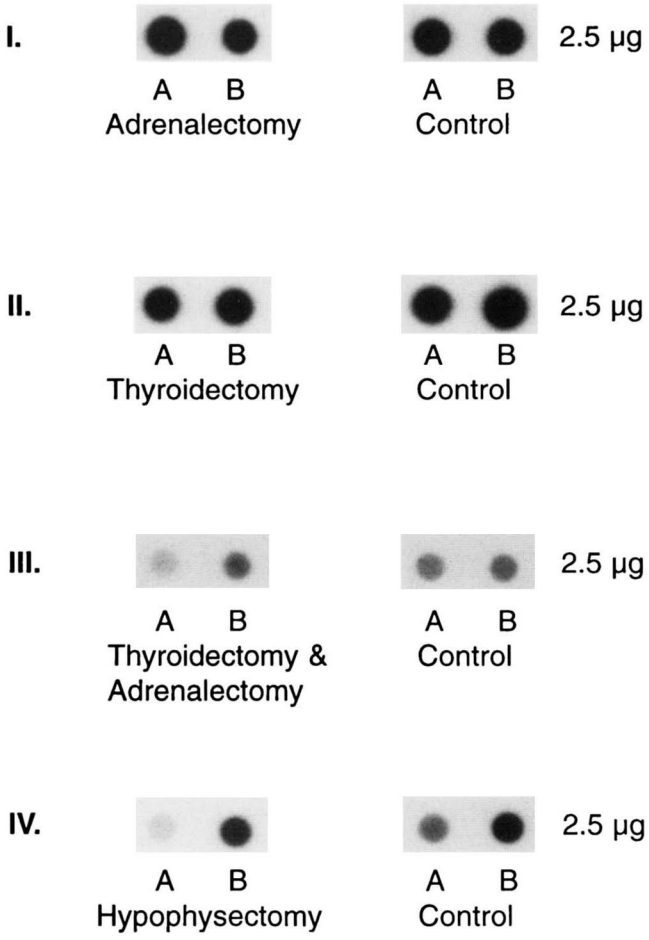

Fig. 5. Representative nuclear run-on assay of cholesterol 7α-hydroxylase (A), and albumin (B). A comparison of the relative transcriptional activity of cholesterol 7α-hydroxylase (A) in adrenalectomy, thyroidectomy, thyroidectomy plus adrenalectomy, and hypophysectomy rats as compared to their sham-operated paired controls is shown in this representative nuclear run-on assay (see Methods). Albumin (B), whose transcriptional activity was unaltered by surgical hormonal manipulation, served as an internal control for changes observed in cholesterol 7α-hydroxylase activity.

(18) found that giving thyroid hormone to hypophysectomized rats led to an increase in cholesterol 7α-hydroxylase mRNA levels within 1 h after its administration; while Gebhard and Prigge (31) found that hypophysectomized rats after receiving thyroid hormone had increased bile acid synthesis rates.

The diurnal variation of cholesterol 7α-hydroxylase activity follows that of circulating hydrocortisone or corticosterone, and is abolished by adrenalectomy or hypophysectomy (32–34). This diurnal variation appears to be mediated at the level of gene transcription (35). Two laboratories have recently identified at least one functional binding site for the diurnally expressed liver-enriched transcription factor, albumin D-element-binding protein (DBP), in the cholesterol 7α-hydroxylase promoter (36, 37).

The synergistic interactions of multiple hormones in the regulation of cholesterol 7α-hydroxylase in vivo have not been studied previously. Multiple mechanisms of transcriptional synergy have been described (38), including cooperative DNA binding of positive *trans*-acting factors to adjacent promoter binding sites (39) or direct interaction of multiple factors with elements of the transcriptional machinery (40, 41). Based upon the recent work of Crestani, Stroup, and Chiang (29), however, this synergy is not likely mediated by cooperative binding of thyroid hormone receptors (TRs) and glucocorticoid receptors (GRs) to the cholesterol 7α-hydroxylase promoter. In transient transfection experiments using cholesterol 7α-hydroxylase promoter–reporter constructs, these authors have observed increased reporter gene activity only after the addition of dexamethasone; the addition of thyroid hormone had no effect. This observation suggests that glucocorticoids stimulate cholesterol 7α-hydroxylase transcription by glucocorticoid/GR complex binding to a glucocorticoid responsive element (GRE). It is not certain whether thyroid hormone functions in a more indirect manner. A recent example of such indirect synergy (42) raises the possibility that thyroid hormone/TR complexes may facilitate glucocorticoid receptor binding to the cholesterol 7α-hydroxylase promoter.

The mechanisms by which glucocorticoids augment cholesterol 7α-hydroxylase activity are likely to be similarly complex. As noted above, functional DBP-binding sites appear to mediate the circadian rhythm of cholesterol 7α-hydroxylase transcription (36, 37). Whether glucocorticoids affect DBP expression or binding to the cholesterol 7α-hydroxylase promoter as the result of the circadian secretion of endogenous glucocorticoids (43) has not been resolved. Although consensus sequences for GR binding have been identified in rat, human, and hamster cholesterol 7α-hydroxylase promoters (44), experimental proof of their ability to bind GRs and stimulate reporter gene expression is not yet available. In primary cultures of rat hepatocytes, the addition of dexamethasone likely increases cholesterol 7α-hydroxylase mRNA half-life as well as transcriptional activity, similar to the mRNAs of other bile acid biosynthetic enzymes (45–47). Furthermore, the addition of high concentrations of dexamethasone (i.e., 10 μM) to cultured rat hepatocytes suppresses cholesterol 7α-hydroxylase mRNA levels (17). Thus, the precise mechanisms of glucocorticoid regulation of cholesterol 7α-hydroxylase activity are still unclear, and probably involve more than "classical" glucocorticoid/GR binding to a GRE.

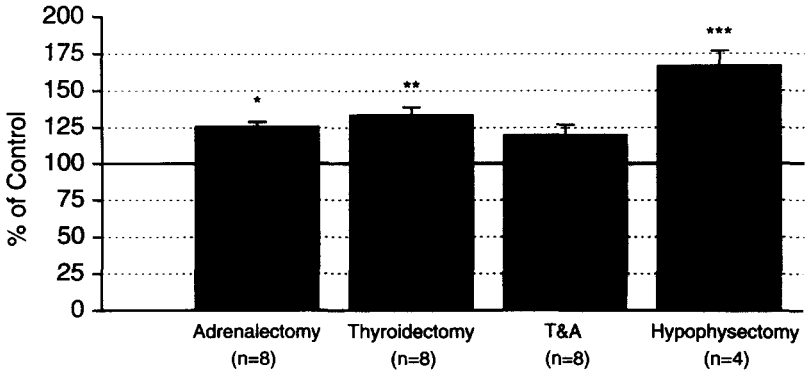

Fig. 6. Effect of adrenalectomy, thyroidectomy, thyroidectomy plus adrenalectomy, and hypophysectomy on hepatic microsomal cholesterol. Shown are hepatic free microsomal cholesterol levels 10 days after surgical hormonal manipulation. Data are expressed as % of sham-operated paired controls (mean ± SE); $*P < 0.01$; $**P < 0.02$; $***P < 0.05$.

In the study of Crestani et al. (29) most effects of physiological stimuli are mediated through elements in the promoter of the cholesterol 7α-hydroxylase gene located in the region downstream of 416 bp; most repressive elements are observed between −344 and −272 bp. The level of cholesterol 7α-hydroxylase gene expression may be determined by binding of different transcription factors to overlying consensus sequences. The localization of *cis*-acting elements within a very short region of the cholesterol 7α-hydroxylase promoter allows for a "cross talk" of different signal transduction pathways, and may be responsible for fine tuning of responses of this important enzyme to a variety of physiologic stimuli.

The observed increases in microsomal cholesterol after endocrine ablation were inversely proportional to changes in cholesterol 7α-hydroxylase specific activity. This finding compliments Ness et al. (18), who reported that in hypophysectomized rats the administration of thyroid hormone alone led to a rapid increase in cholesterol 7α-hydroxylase mRNA levels followed by temporally related increases in LDL receptor, apoA-I, and HMG-CoA reductase mRNA levels, respectively. However, at more physiologic concentrations of thyroid hormone, maximal stimulation of cholesterol 7α-hydroxylase occurred with little or no effect on the other three parameters. Cholesterol feeding or mevalonate infusion in rats is known to up-regulate cholesterol 7α-hydroxylase. The inverse relationship between microsomal cholesterol and cholesterol 7α-hydroxylase in our studies indicates that decreased cholesterol 7α-hydroxylase may be responsible for decreased hepatic cholesterol metabolism, leading to cholesterol accumulation.

We conclude that thyroid hormone, glucocorticoids, and possibly an as yet unidentified pituitary hormone play a role in maintaining basal cholesterol 7α-hydroxylase gene expression in the rat. The molecular mechanisms by which thyroid hormone, glucocorticoids, and possibly growth hormone regulate expression of cholesterol 7α-hydroxylase remain important areas for future investigation.

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