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Thyroid Hormone Rapidly Induces Hepatic LDL Receptor mRNA Levels in Hypophysectomized Rats[1]

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The effects of hypophysectomy and thyroid hormone treatment on the expression of the hepatic LDL receptor gene was studied in young male rats. Hypophysectomy lowered levels of LDL receptor mRNA and protein. It was found that increased levels of LDL receptor mRNA could be detected 30 min after giving hypophysectomized rats an intramuscular injection of 10 μg of L-triiodothyronine (T_3) per 100 g of body weight. This dose of T_3 also increased hepatic LDL receptor protein levels within 1 h. A T_3 dose of only 0.25 μg per 100 g increased LDL receptor mRNA levels more than threefold. The half-life of the hepatic LDL receptor mRNA was found to be about 30 min and was unaffected by T_3. The data suggests that thyroid hormone acts physiologically to induce hepatic LDL receptor expression. This action may explain, in part, the hypocholesterolemic effect of the hormone.

Key Words: **thyroid hormone; LDL receptor; Northern Blotting; mRNA half-life; immunoblotting.**

The hepatic LDL receptor plays a key role in maintaining cholesterol homeostasis (1). About 70% of the body's total LDL receptors are expressed in liver (2). The liver is the principle site for elimination of cholesterol by conversion to bile acids and by secretion of cholesterol in the bile. Hypocholesterolemic drugs such as Lovastatin, which are potent inhibitors of HMG-CoA reductase, are thought to act by inducing hepatic LDL receptors (3). Feedback regulation of LDL receptor gene expression by cholesterol has been studied extensively (4, 5). Much is known concerning the mechanism by which this regulation occurs. A 10-bp sterol response element has been identified (4) and a protein which binds to it and thereby controls transcription has been purified and cloned (6, 7). In contrast, much less information exists concerning the regulation of hepatic LDL receptor expression by hormones. It has been reported that pharmacological doses of estrogen, in the form of ethynylestradiol, increase hepatic LDL receptor activity and mRNA levels (8, 9). Administration of growth hormone was found to increase hepatic LDL receptor mRNA levels about two-fold and enhance the stimulation of hepatic LDL receptor mRNA by estrogen (9). Stimulation of endogenous growth hormone secretion in aging rats by giving growth hormone releasing hormone and growth hormone releasing hexapeptide also significantly increased hepatic LDL receptor mRNA levels (10). In terms of thyroid hormone, it has been reported that administration of L-triiodothyronine (T_3)[3] to hypophysectomized rats increases LDL receptor mRNA levels within 6 h (11). Studies at earlier time points or at physiological doses of T_3 were not carried out. In the present study we examined the effects of T_3 on hepatic LDL receptor mRNA expression 30 min to 2 h after giving the hormone and investigated the effects of physiological doses of the hormone. In addition, the effects on LDL receptor immunoreactive protein levels and the half-life of the mRNA were determined.

MATERIALS AND METHODS

Materials. The cDNA probe for the rat liver LDL receptor, LDLR-1, was a generous gift from Dr. Richard Tanaka (12). The cDNA probes for human glyceraldehyde 3-phosphate dehydrogenase, pHcGAP, and

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[3] Abbreviations used: T_3, L-triiodothyronine; H and Hypx, hypophysectomized; GAPD, glyceraldehyde 3-phosphate dehydrogenase; LDLR, LDL receptor; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; SDS, sodium dodecyl sulfate.

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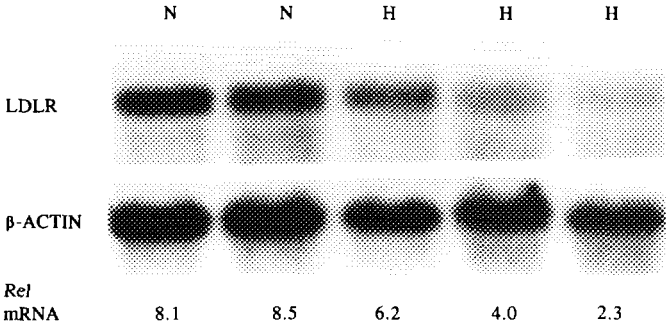

FIG. 1. Effect of hypophysectomy on hepatic LDL receptor mRNA levels. Poly(A)$^+$ RNA isolated from normal (N) and hypophysectomized (H) rats was probed for LDL receptor and β actin. Ten micrograms of Poly(A)$^+$ RNA was applied to each lane. The relative levels of *LDL* receptor mRNA are presented.

β-actin, HHCI89, used as internal standards, were obtained from American Type Culture Collection. Gene Screen Plus membranes and [α-^{32}P]dCTP were purchased from Dupont/New England Nuclear. The nick translation kit, oligo(dT) cellulose and actinomycin D were purchased from Boehringer Mannheim. TRI Reagent was from Molecular Research Center. T_3 was purchased from Sigma Chemical.

Animals. Hypophysectomized male Sprague–Dawley rats weighing 125 to 150 g were purchased from Harlan Industries (Madison, WI). They were housed in a light-controlled room with a 14-h light period followed by a 10-h dark period. They were fed Purina Rodent Laboratory Chow 5001 and water *ad libitum*. T_3 solutions in saline containing 10 mg/ml bovine serum albumin adjusted to pH 11 with sodium hydroxide were prepared. T_3 was given as a single intramuscular injection to hypophysectomized rats 14 to 21 days following surgery. Actinomycin D was given subcutaneously at a dose of 120 μg per 100 g. All rats were killed at the fifth hour of the dark cycle by decapitation.

mRNA isolation and Northern blotting analysis. Total RNA was isolated from fresh rat liver using the acid guanidinium thiocyanate–phenol–chloroform extraction method (13). For this method, TRI Reagent was used as it is formulated as a single convenient reagent. Thus a 0.2-g piece of liver was quickly removed, placed in 2 ml of room-temperature TRI Reagent, and immediately homogenized. After 5 min, 0.4 ml of chloroform was added and the sample mixed vigorously. The mixture was then centrifuged at 12,000*g* for 15 min at 4°C. The aqueous phase was removed and 1 ml of isopropanol was added. The sample was then mixed and allowed to remain at room temperature for 5 to 10 min. It was then centrifuged at 12,000*g* for 10 min at 4°C. The total RNA pellet was dissolved in 100 μl of diethylpyrocarbonate treated water and the poly(A)$^+$ RNA was isolated by oligo(dT) cellulose chromatography (14). Ten-microgram aliquots of poly(A)$^+$ RNA were denatured and electrophoresed on 1% agarose gels containing 3% formaldehyde. The separated RNAs were transferred to Gene Screen Plus membranes and baked at 80°C under vacuum for 2 h to fix the RNA to the filters. The cDNA probes were labeled with ^{32}P by nick translation to specific activities *ranging from* 2×10^8 *to* 3×10^9 *cpm/μg. Hybridizations and washings* were performed as previously described (15). Autoradiography was performed at −70°C for 4 to 48 h. Several exposures of each blot were done and scanned with an LKB Ultrascan laser densitometer to determine relative levels of LDL receptor mRNA. All values were corrected for the amount of glyceraldehyde 3-phosphate dehydrogenase (GAPD) or β-actin mRNA present.

Immunoblotting analysis. Levels of hepatic LDL receptor protein were determined by immunoblotting analysis. A polyclonal antisera to rat hepatic LDL receptor was generated in rabbits. A peptide corresponding to a portion of the C-terminal region of the protein which extends into the cytoplasm was synthesized. This peptide, val–asp–glu–leu–ser–val–met–asn–arg–ser–pro–tyr–(cys), was conjugated via the added cysteine residue to keyhole limpet hemocyanin using Imject maleimide-activated keyhole limpet hemocyanin from Pierce Chemical. A rabbit was injected at multiple sites along the back intradermally with 2 mg of the conjugate in 4 ml of Freund's Complete Adjuvant. Two weeks later, a 1-mg booster of the conjugate in Freund's Incomplete Adjuvant was given. After an additional 2 weeks, the rabbit was bled from the marginal ear vein and sera was prepared and used for immunoblotting analysis. Rat liver microsomal membranes were prepared as previously described (16). These membranes were subjected to SDS–polyacrylamide gel electrophoresis on 7.5% gels. The proteins were electrophoretically transferred to Immobilon membranes. The membranes were blocked with 3% gelatin and then incubated with a 1:375 dilution of the rabbit anti-rat LDL receptor sera. LDL receptor immunoreactive protein was detected using an alkaline phosphatase-conjugated second antibody (17). *The size of the LDL receptor protein was determined* from a semilog plot of migration of known proteins.

RESULTS

The effect of hypophysectomy on hepatic LDL receptor mRNA levels was examined by Northern blotting anal-

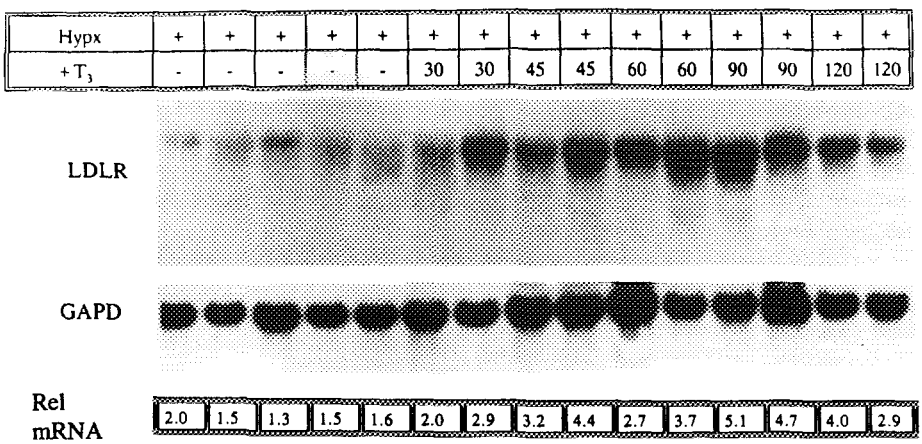

FIG. 2. Time course for induction of hepatic LDL receptor mRNA by T_3. Hypophysectomized rats were injected with T_3 (10 μg per 100 g) and killed 30 to 120 min later. The Northern blot was probed for LDL receptor and GAPD.

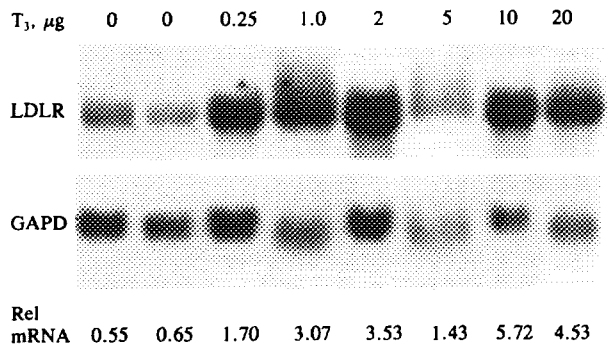

FIG. 3. Dose curve for induction of hepatic LDL receptor mRNA by T_3. Hypophysectomized rats were injected with 0.25 to 20 μg per 100 g of T_3 2 h prior to being killed. The blot was probed for LDL receptor and GAPD. The relative level of LDL receptor mRNA is given. The 5-μg point is atypical.

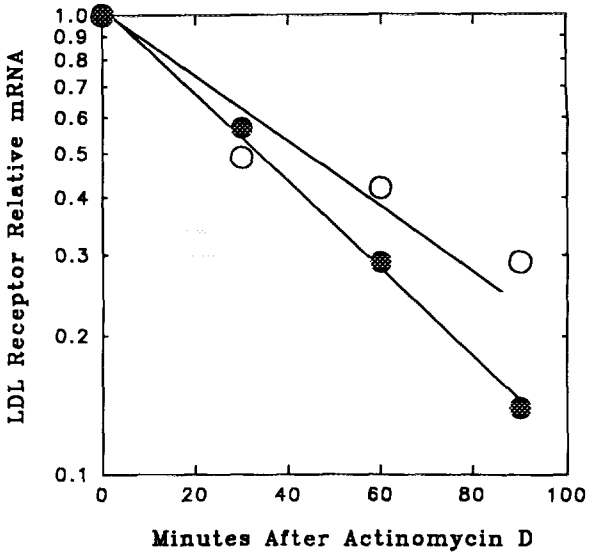

FIG. 5. Determination of the half-life of the hepatic LDL receptor mRNA. Values corrected for β-actin were plotted as a fraction of their zero time controls for H (○) and H + T_3 (●) rats.

ysis. As shown in Fig. 1, these levels were reduced by about 50% by hypophysectomy. This is in agreement with the previously reported decreases in LDL receptor binding activity in liver membranes from hypophysectomized rats (9).

To evaluate the physiological significance of the previously reported T_3-mediated increase in hepatic LDL receptor mRNA levels (11), time and dose–response studies were carried out. Hypophysectomized rats were selected as the hormone-deficient model since possible interference from other pituitary controlled hormones would be avoided. As shown in Fig. 2, administration of T_3 caused a rapid increase in LDL receptor mRNA levels. Increases began as soon as 30 min after T_3 treatment and reached maximal levels within 90 min. A typical dose–response curve is presented in Fig. 3. Greater than a 3-fold increase in LDL receptor mRNA levels can be obtained with a dose of 0.25 μg per 100 g of body weight. This is the dose required to obtain 50% saturation of rat liver nuclear T_3 receptors for 4 h when given intramuscularly (18). Higher doses of T_3 produced 8- to 10-fold increases. Relative LDL receptor mRNA levels corrected for GAPD are presented. These observations suggest that the T_3 mediated increase in LDL receptor gene expression may be physiological. Based on the rapid time course it may also be a primary effect of the hormone.

To determine whether the T_3-induced increase in LDL receptor mRNA levels was due to stabilization of the mRNA, half-life determinations were carried out. Actinomycin D was given 1 h after T_3 to block further RNA synthesis and the rate of LDL receptor mRNA disappearance was determined by Northern blotting analysis (Fig. 4). After probing for LDL receptor mRNA, the filter was stripped and reprobed for β-actin. Thus relative LDL receptor mRNA values were determined. These were plotted as shown in Fig. 5 to obtain half-lives for the mRNA from T_3-treated and untreated hypophysectomized rats. The half-life was essentially unchanged by T_3 treatment.

Since levels of mRNA do not always correlate with protein levels, the effects of hypophysectomy and T_3 treatment on hepatic LDL receptor protein levels were examined using immunoblotting analysis. As can be seen in Fig. 6, normal young male rats express significant levels of hepatic LDL receptor protein. Upon hypophysectomy these levels drop. Administration of T_3 rapidly restores these levels to those seen in the normal animals. Thus, levels of immunoreactive hepatic LDL receptor protein change in parallel with the mRNA. The size of the hepatic LDL receptor was found to be about 155 kDa. Feeding 2% cholesterol to these animals for 4 days essentially had no effect on levels of LDL receptor protein. This is in agreement with a lack of change in hepatic LDL receptor mRNA levels.[4]

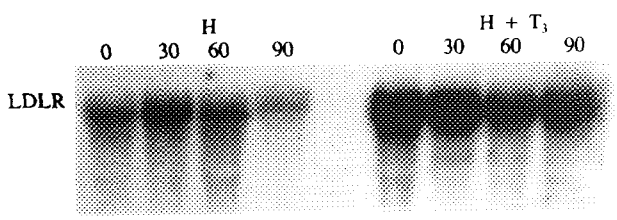

FIG. 4. Degradation of hepatic LDL receptor mRNA. Hypophysectomized (H) and T_3-treated hypophysectomized (H + T_3) rats were given actinomycin D (120 μg/100 g) and killed 30 to 90 min later. Rats received T_3 (10 μg per 100 g) 1 h prior to actinomycin D. The amount of LDL receptor mRNA remaining was determined by Northern blotting analysis. The blot was stripped and reprobed for β-actin.

[4] Ness, G. C., unpublished observations.

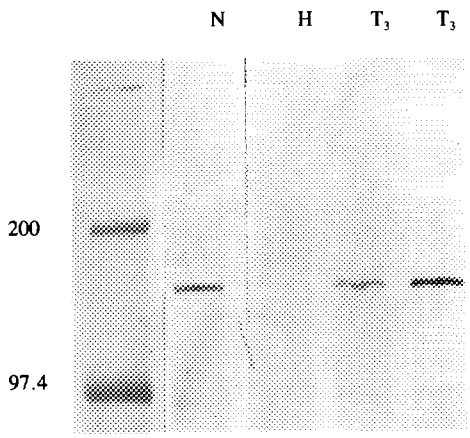

FIG. 6. Effect of hypophysectomy and T_3 treatment on levels of hepatic LDL receptor protein. An immunoblot of 300 μg of microsomal protein from normal (N), hypophysectomized (H), and T_3-treated hypophysectomized (T_3) rat liver is shown. Protein standards are shown on the left side. Hypophysectomized rats were given 10 μg per 100 g of T_3 1 h prior to being killed.

DISCUSSION

The finding that T_3 at doses as low as 0.25 μg per 100 g could significantly increase hepatic LDL receptor gene expression suggests that this effect may be physiological and of central importance to the well known hypocholesterolemic effect of the hormone (19). The magnitude of the increase seen with this dose of T_3 is essentially the same as observed for cholesterol 7-α-hydroxylase mRNA (20). It would seem that T_3 acts to enhance cholesterol uptake by the liver and its subsequent elimination via conversion to bile acids. At these doses of T_3 and within this time frame, no effect on HMG-CoA reductase gene expression is observed (20). The very rapid increase in hepatic LDL receptor mRNA caused by T_3 is indicative of a primary effect. This same rapid time course was also observed for the effect of T_3 on cholesterol 7-α-hydroxylase gene expression (20).

The relatively high level of expression of hepatic LDL receptor mRNA and protein observed in the young rats used in this study may be important to their known resistance to dietary cholesterol. As rats age, their serum cholesterol levels rise markedly, they have lower serum T_3 levels and their hepatic LDL receptor mRNA significantly decrease (10). Rats also lose their resistance to dietary cholesterol upon hypophysectomy (21). Based on the data presented in this study, decreased expression of the hepatic LDL receptor is a likely explanation. Hypophysectomized rats exhibit markedly elevated serum cholesterol levels.[4] Adequate T_3 levels may be central to maintaining cholesterol homeostasis.

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