Hormonal regulation of acid cholesterol ester hydrolase activity: effects of triiodothyronine and 17α-ethynylestradiol

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Cholesterol ester hydrolase activity was measured in isolated rat hepatocytes and adipocytes. Administration of triiodothyronine to rats resulted in a specific and selective increase in lysosomal acid (pH 4.5) cholesterol ester hydrolase activity in hepatocytes. Since the majority of lipoprotein degradation occurs in liver parenchymal cells (hepatocytes), the stimulation of liver (hepatocyte) acid cholesterol ester hydrolase activity by triiodothyronine could contribute to the hypocholesterolemic action of thyroid hormones. Treatment of rats with 17α-ethynylestradiol to increase the hepatic degradation of lipoproteins did not change acid cholesterol ester hydrolase activity in liver, indicating that the thyroid hormone induced stimulation of acid cholesterol ester hydrolase activity in hepatocytes is not a secondary effect owing to the increased hepatic catabolism of low density lipoproteins (LDL). In contrast to the results with hepatocytes, hyperthyroidism did not increase acid cholesterol ester hydrolase activity in rat adipocytes.

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On a déterminé l'activité hydrolasique des esters cholestériques dans les hépatocytes et les adipocytes isolés de rats. L'administration de triiodothyronine aux rats provoqua une augmentation spécifique et sélective de l'activité hydrolasique des esters cholestériques acides (pH 4,5) lysosomiaux des hépatocytes. La dégradation des lipoprotéines se produisant majoritairement dans les cellules parenchymateuses (hépatocytes), la stimulation de l'activité hydrolasique des esters cholestériques acides (hépatocyte) du foie par la triiodothyronine pourrait contribuer à l'action hypocholestérolémique des hormones thyroïdiennes. Le traitement des rats avec du 17α-éthynyloestradiol pour augmenter la dégradation hépatique des lipoprotéines ne modifia pas l'activité hydrolasique des esters cholestériques acides du foie; ceci indique que la stimulation de l'activité hydrolasique des esters cholestériques acides, induite dans les hépatocytes par les hormones thyroïdiennes, n'est pas un effet secondaire résultant d'une augmentation du catabolisme hépatique des lipoprotéines de basse densité (LDL). Contrairement aux résultats obtenus avec les hépatocytes, l'hyperthyroïdisme n'augmenta pas l'activité hydrolasique des esters cholestériques acides des adipocytes de rats.

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Introduction

Hypothyroidism results in a decrease in the rates of low density lipoprotein (LDL) catabolism (Sykes et al. 1981; Katz-Feigenbaum et al. 1981); this decrease in lipoprotein degradation can be prevented by the administration of thyroid hormones (Katz-Feigenbaum et al. 1981). Furthermore, it has been shown that thyroid hormones specifically stimulate the receptor-mediated catabolism of LDL (Katz-Feigenbaum et al. 1981), which involves the binding of LDL to cell-surface receptors, internalization of LDL, and the lysosomal degradation of the cholesterol ester component of the lipoprotein particle by an acid cholesterol ester hydrolase (Brown et al. 1981). In rats, the liver accounts for approximately two-thirds of the total degradation of LDL; kidney and adipose tissue represent some of the important extrahepatic catabolic sites (Pittman et al. 1982). Recently, the administration of triiodothyronine to rats has been shown to increase lysosomal acid cholesterol ester hydrolase activity in liver (Katz-Feigenbaum et al. 1981; Severson and Fletcher 1981), kidney (Katz-Feigenbaum et al. 1981), and epididymal fat pads (Severson and Fletcher 1981). These results suggest that the stimulation of lysosomal acid cholesterol ester hydrolase activity by thyroid hormones at several tissue sites may be linked to increases in the catabolism of LDL (Wolinsky 1980), and thus could contribute to the hypocholesterolemic effect of thyroid hormones. The effects of thyroid hormones on acid cholesterol ester hydrolase activity were tissue specific as the induction of hypothyroidism and the administration of triiodothyronine did not influence acid cholesterol ester hydrolase activity in the heart (Severson and Fletcher 1981), a tissue that does not contribute significantly to the catabolism of LDL (Pittman et al. 1982).

The majority (estimates ranging from 65 to 92%) of the degradation of lipoproteins by the liver has been reported to occur in parenchymal cells (hepatocytes) (Pittman et al. 1982; Groot et al. 1981; Lippiello et al. 1981; Van Tol and Van Berkel 1980). Kupffer cells, for example, do not possess a high-affinity binding site for LDL (Goldstein et al. 1979), and more than 85% of the receptor-independent catabolism of methylated LDL has also been attributed to hepatocytes (Carew et al. 1982). However, the specific activity of acid cholesterol ester hydrolase has been reported to be more than 10-fold higher in nonparenchymal cells than in parenchymal cells from liver (Van Berkel et al. 1980) so that approximately one-half of the total acid cholesterol ester hydrolase activity in liver is present in nonparenchymal cells. Clearly, if the effect of thyroid hormones on liver acid cholesterol ester hydrolase activity (Katz-Feigenbaum et al. 1981; Severson and Fletcher 1981) is to have the potential for physiological significance in relation to lipoprotein degradation (Wolinsky 1980), then it is important to show that thyroid hormones can regulate the acid cholesterol ester hydrolase in isolated hepatocytes. Similarly, the binding, internalization, and degradation of LDL has only been reported in isolated adipocytes (Angel et al. 1979), but the effects of thyroid hormone on acid cholesterol ester hydrolase activity were observed with preparations from epididymal fat pads (Severson and Fletcher 1981). Therefore, the objective of this investigation was to establish if thyroid hormones could increase acid cholesterol ester hydrolase activity in isolated rat

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hepatocytes and adipocytes. The specificity of the hormonal regulation of liver acid cholesterol ester hydrolase by thyroid hormones was also examined by determining the effect of estrogens on liver acid cholesterol ester hydrolase activity, since the administration of 17α-ethynylestradiol to rats has also been shown to stimulate the hepatic degradation of LDL owing to an increase in LDL receptors (Chao et al. 1979; Kovanen et al. 1979).

Experimental procedures

Male Sprague–Dawley rats were treated with subcutaneous injections of triiodothyronine (0.5 mg/kg body weight; Calbiochem, A grade) for 3 days as described previously (Severson and Fletcher 1981). Hepatic parenchymal cells were isolated using Seglen's collagenase perfusion technique (Seglen 1976). Following a 10- to 30-min digestion with 0.33 mg/mL collagenase (Worthington, type II) in Krebs–Henseleit bicarbonate buffer, the liver was minced and incubated for 10 min with 60 mL of oxygenated Krebs–Henseleit bicarbonate buffer which contained 1% bovine albumin. The dispersed cells were filtered through a 100-mesh nylon screen and washed three times; viability, determined by 0.4% trypan blue exclusion, was greater than 90%. Hyperthyroidism had no effect on the yield or viability of hepatocytes. Adipocytes were prepared with minor modifications to the method described by Rodbell (1964). Hepatocytes and adipocytes were homogenized in an isotonic sucrose buffer (0.25 *M* sucrose – 1 m*M* EDTA – 10 m*M* HEPES, pH 7.5) at 4°C for 10–20 s with a Polytron PT-10 homogenizer (Severson and Fletcher 1981). Adipocyte homogenates were centrifuged at 1000 × g and the resulting infranatant fraction was used for enzyme assays.

In some experiments, rats were treated for 5 days with 17α-ethynylestradiol (10 mg/kg body weight; Sigma Chemical Co.) as described by Chao et al. (1979). Livers were removed, homogenized as described previously (Severson and Fletcher 1981), and then centrifuged at 1000 × g for 20 min to obtain a low-speed supernatant fraction. Serum samples collected from these rats were analyzed for cholesterol by the spectrophotometric procedure outlined in the Sigma Technical Bulletin No. 350.

Glycerol-dispersed cholesterol oleate substrates were prepared as described by Severson and Fletcher (1981). Routinely, appropriate quantities of cholesterol [1-^{14}C]oleate and lecithin were dried under N_2 and then dispersed into anhydrous glycerol with a Polytron homogenizer. Aliquots from this stock solution were then diluted with the appropriate buffer and cholesterol ester hydrolase activity was measured as described previously (Severson and Fletcher 1981); assays were linear with respect to time and protein.

Protein (Robrish et al. 1978), DNA (Thomas and Farquhar 1978), and the lysosomal marker enzyme *N*-acetylglucosaminidase (Peters et al. 1972) were measured by fluorometric techniques. Cholesterol ester hydrolase activity was calculated as both units per milligram protein and as units per milligram DNA; units of enzyme activity were arbitrarily defined as given in Severson and Fletcher (1981). The DNA content of adipocyte homogenates was not detectable; consequently, enzyme activity in adipocyte preparations is expressed only as units per milligram protein.

Results

Cholesterol ester hydrolase activity, determined with a glycerol-dispersed cholesterol oleate substrate preparation containing lecithin, in a low-speed supernatant (1000 × *g*) fraction from hepatocytes had an acid pH optimum of 4.5. A pH optimum of 4.0 has been reported for hepatocyte homogenates by Van Berkel et al. (1980); this discrepancy is likely due to differences in the substrate preparations. Some cholesterol ester hydrolase activity could also be measured at neutral and alkaline pH values, but this was much less (one-third to one-quarter) than the acid activity. Acid cholesterol ester hydrolase activity (measured at pH 4.5) in subcellular fractions obtained by differential centrifugation of hepatocyte homogenates was localized to particulate fractions and the distribution was very similar to that for *N*-acetylglucosaminidase, a lysosomal marker enzyme (results not shown). Nilsson et al. (1973) have concluded previously that the acid cholesterol ester hydrolase in hepatocytes was of lysosomal origin. In contrast to the acid cholesterol ester hydrolase, the neutral cholesterol ester hydrolase (assayed at pH 7) was localized in the soluble fraction after centrifugation at 100 000 × *g*.

The pH dependency of cholesterol ester hydrolase in isolated adipocytes was similar to that observed in epididymal fat pad preparations (Severson and Fletcher 1981), in that although enzyme activity could be measured at acid pH values, a distinct acid pH optimum was obscured by the considerable activity in the neutral–alkaline pH range. Consequently, acid cholesterol ester hydrolase activity in adipocyte infranatant preparations was also routinely determined at pH 4.5.

Previous results (Severson and Fletcher 1981) have reported that acid cholesterol ester hydrolase activity was reduced in liver preparations from thyroidectomized rats, indicating that physiological levels of thyroid hormones can regulate the acid cholesterol ester hydrolase activity. However, since hypothyroidism (thyroidectomized rats) resulted in a marked decrease in liver wet weight and liver weight as a percentage of body weight (Severson and Fletcher 1981), it was decided to measure acid cholesterol ester hydrolase activity only in hepatocytes isolated from control and hyperthyroid rats. The administration of triiodothyronine to euthyroid control rats for 3 days produced a two- to three-fold increase of acid cholesterol ester hydrolase activity in hepatocyte homogenates (Table 1); this result extends the previous observation that the treatment of control or hypothyroid rats with triiodothyronine produced an increase in acid cholesterol ester hydrolase activity in whole liver preparations (Severson and Fletcher 1981). Hyperthyroidism did not change either *N*-acetylglucosaminidase (Table 1) or neutral cholesterol ester hydrolase (results not shown) activity in these hepatocyte preparations.

The specificity of the thyroid hormone-induced increase in acid cholesterol ester hydrolase activity in liver (Katz-Feigenbaum et al. 1981; Severson and Fletcher 1981) and hepatocyte (Table 1) preparations was further examined by determining if the increased hepatic catabolism of LDL owing to the administration of estrogens (Chao et al. 1979; Kovanen et al. 1979) was also associated with an increase in acid lysosomal cholesterol ester hydrolase activity. Preparations from the whole liver were studied to determine if estrogens might influence cholesterol ester hydrolase activity in either nonparenchymal cells and (or) hepatocytes, and to avoid any potential effects of the experimental treatment on the isolation of hepatocytes. As shown in Table 2, administration of pharmacologic doses of estrogens (17α-ethynylestradiol) for 5 days resulted in a reduction in total serum cholesterol to 16% of control levels but had no effect on liver acid cholesterol ester hydrolase activity.

Previously it was reported that thyroidectomy resulted in a decrease in acid cholesterol ester hydrolase activity in rat epididymal fat pad preparations, and that the administration of triiodothyronine to both control and hypothyroid rats increased fat pad acid hydrolase activity when results were expressed as units per milligram DNA (Severson and Fletcher 1981). In contrast, hyperthyroidism had no significant effect on either acid cholesterol ester hydrolase or *N*-acetylglucosaminidase activity in isolated adipocytes (Table 1). Furthermore, the ad-

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TABLE 1. Effect of triiodothyronine on acid cholesterol ester hydrolase and *N*-acetylglucosaminidase activity in isolated rat hepatocytes and adipocytes

Preparation	*n*	Acid cholesterol ester hydrolase		*N*-acetyl-glucosaminidase
		U/mg protein	U/mg DNA	U/mg protein
Hepatocytes control	4	13.0±0.8	358±39	0.30±0.07
Triiodothyronine-treated	4	37.3±3.0**	691±76*	0.40±0.01
Adipocytes control	13	33.6±4.1	—	0.08±0.01
Triiodothyronine-treated	13	38.9±4.8	—	0.08±0.01

NOTE: Hydrolase activity was measured at pH 4.5. Results are expressed as the mean ± SE. A significant difference from the appropriate control is indicated by *, $p < 0.01$; **, $p < 0.001$.

TABLE 2. Effect of 17α-ethynylestradiol on acid cholesterol ester hydrolase activity in rat liver

Experimental group		Serum cholesterol	Acid cholesterol ester hydrolase	
		mg/dL	U/mg protein	U/mg DNA
Control	(4)	69±6	6.4±0.8	255±33
Estrogen-treated	(4)	11±2**	7.6±0.3	174±27

NOTE: Hydrolase activity was measured at pH 5. Results are expressed as the mean ± SE for the number of preparations indicated in parentheses. A significant difference from the appropriate control is indicated by **, $p < 0.001$.

ministration of triiodothyronine had no significant effect on neutral cholesterol ester hydrolase activity measured at pH 7.5 (control, 69.6 ± 10.3 U/mg protein; hyperthyroid, 79.8 ± 12.5 U/mg protein; $n = 12$).

Discussion

The hepatic degradation of lipoproteins is inhibited by chloroquine, indicating that lysosomal enzymes are involved in the catabolic process (Stein et al. 1977; Floren and Nilsson 1977). The hydrolysis of cholesterol esters bound to lipoproteins and lipoprotein remnants by the lysosomal acid cholesterol ester hydrolase provides a source of exogenous cholesterol that inhibits the biosynthesis of cholesterol through a reduction in 3-hydroxy-3-methylglutaryl CoA reductase activity (Lakshmanan et al. 1981). Thus, regulation of acid cholesterol ester hydrolase activity may play an important role in the hepatic metabolism of cholesterol.

Since the liver is the major organ involved in the degradation of LDL and as the majority of this hepatic catabolism of lipoproteins occurs in parenchymal cells (hepatocytes) (Pittman et al. 1982), it was important to demonstrate that the increase in acid cholesterol ester hydrolase activity observed in whole liver preparations after the administration of triiodothyronine to rats (Katz-Feigenbaum et al. 1981; Severson and Fletcher 1981), could also be observed in hepatocyte preparations if this change in tissue (cellular) metabolic activity is to be linked to the dynamic clearance of lipoproteins (Wolinsky 1980). This question is particularly relevant given that acid cholesterol ester hydrolase activity is very high in the nonparenchymal cells (Van Berkel et al. 1980) that do not contribute substantially to the hepatic degradation of lipoproteins (Pittman et al. 1982; Groot et al. 1981; Lippiello et al. 1981; Van Tol and Van Berkel 1980; Goldstein et al. 1979; Carew et al. 1982). Hyperthyroidism did produce an increase in lysosomal acid cholesterol ester hydrolase activity in hepatocytes, which was specific since triiodothyronine did not increase the activity of *N*-acetylglucosaminidase, a lysosomal marker enzyme. This suggests that the thyroid hormone-induced increase in *N*-acetylglucosaminidase activity observed in whole liver (Severson and Fletcher 1981) may have been restricted to nonparenchymal cells. Hyperthyroidism also did not influence the activity of the neutral cholesterol ester hydrolase in hepatocytes.

Thyroid hormones specifically enhance the receptor-dependent degradation of LDL (Katz-Feigenbaum et al. 1981), probably by increasing the number of high-affinity LDL receptors (Brown et al. 1981). Therefore, the stimulation of the lysosomal acid cholesterol ester hydrolase in hepatocytes by triiodothyronine could be part of a coordinated response of the liver which also includes effects on the hepatic catabolism of cholesterol (Miettenen 1968). The regulatory significance of these thyroid hormone-induced effects will depend on the rate-limiting step in the sequence of biochemical events involved in the hepatic catabolism of LDL and cholesterol. It could be argued that the effect of triiodothyronine on lysosomal acid cholesterol ester hydrolase activity in hepatocytes might be a secondary or compensatory response to the increased receptor-dependent degradation of LDL owing to the increased number of LDL receptors on the hepatocyte (Katz-Feigenbaum et al. 1981; Brown et al. 1981). This possibility was tested by determining if the administration of estrogens to rats would also increase liver acid cholesterol ester hydrolase activity, as estrogens also stimulate the hepatic degradation of LDL as a consequence of an increase in high-affinity binding sites on liver cell membranes (Chao et al. 1979; Kovanen et al. 1979). Since the treatment of rats for 5 days with 17α-ethynylestradiol did not change acid cholesterol hydrolase activity in liver preparations despite a reduction in plasma cholesterol levels, this may be taken as additional evidence that the thyroid hormone-induced response is specific and selective. Although Chait et al. (1979) have reported that the addition of triiodothyronine to the culture medium increased LDL binding and degradation in cultured fibroblasts, to date the presence of varying concentrations of triiodothyronine in the incubation or culture medium has not produced an *in vitro* effect on acid cholesterol ester hydrolase activity in either freshly isolated or cultured hepatocytes (T. Fletcher and L. J. Hayden, unpublished observations).

A variety of extrahepatic tissues also contribute to the degradation of LDL (Pittman et al. 1982). Adipocytes have been shown to bind and degrade LDL (Angel et al. 1979), but the administration of triiodothyronine did not increase the acid cholesterol ester hydrolase activity in isolated rat adipocytes. It should be noted that the specific activity of the acid cholesterol ester hydrolase in adipocytes (Table 1) is much less than in

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intact fat pads (Severson and Fletcher 1981), which would be consistent with a substantial amount of enzyme activity being present in stromal-vascular cells. Therefore, thyroid hormone-induced increases in acid hydrolase activity previously observed in epididymal fat pad preparations (Severson and Fletcher 1981) probably occurred in cell types other than adipocytes. Consequently, this effect on the fat pad acid cholesterol ester hydrolase would probably not contribute to any enhancement of LDL degradation owing to thyroid hormones. The assessment of the physiological significance of increases in acid hydrolase in the aorta and kidney in response to thyroid hormones (Katz-Feigenbaum et al. 1981) will require additional experimentation.

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