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EFFECT OF THYROID HORMONES ON ACID CHOLESTEROL ESTER HYDROLASE ACTIVITY IN RAT LIVER, HEART AND EPIDIDYMAL FAT PADS

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The regulation of acid cholesterol ester hydrolase activity by thyroid hormones was studied in subcellular fractions from rat liver, heart, and epididymal fat pads; hydrolase activity was determined at pH 5 with a glycerol-dispersed cholesterol oleate substrate preparation. Acid cholesterol ester hydrolase activity was decreased in liver preparations from thyroidectomized rats relative to activity in livers from euthyroid control rats. Administration of triiodothyronine to either euthyroid or hypothyroid (thyroidectomized) rats resulted in an increase in acid cholesterol ester hydrolase activity in liver preparations. Similar effects of thyroidectomy and the administration of triiodothyronine on acid cholesterol ester hydrolase activity were observed with fat pad preparations. In contrast, no effect of thyroid hormones was observed on acid cholesterol ester hydrolase activity in heart. These results suggest that thyroid hormones may regulate the catabolism of serum lipoproteins, in part, by alterations in lysosomal acid cholesterol ester hydrolase activity in liver and epididymal fat pads.

Introduction

A number of studies have now established that lysosomal function can be influenced by hormones. For example, rates of protein degradation (proteolysis) in rat skeletal muscle were decreased after hypophysectomy and could be increased to control levels by treatment of the rats with thyroid hormones [1]. The mechanism by which thyroid hormones influence proteolysis may, in part, be due to changes in the cellular activity of lysosomal proteases [2]. Thyroidectomy resulted in a decrease in cathepsin B and D activities to approximately 50% of control activities in skeletal muscle and liver homogenates; treatment of hypophysectomized rats with thyroid hormones (L-thyroxine or L-triiodothyronine) produced a 2–3-fold increase in the activities of cathepsins B and D [2].

Thyroid hormones have also been reported to influence acid lipase activity in rat liver [3,4]; thyroxine treatment produced an increase in acid lipase activity (measured at pH 4 with a fluorometric assay using 4-methylumbelliferyl oleate as substrate) whereas thyroidectomy resulted in a decrease in acid lipase activity. Lysosomal acid cholesterol ester hydrolases have been implicated in the degradation of the cholesterol ester component of internalized lipoproteins in liver [5–7] as well as in peripheral (non-hepatic) tissues [7–9]. Consequently, effects of thyroid hormones on acid cholesterol ester hydrolase activity could account, in part, for the observations that human hypothyroidism is characterized by hypercholesterolemia whereas hyperthyroidism is associated with hypocholesterolemia [10–12]. The present investigations were undertaken to determine

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; TSH, thyrotropin; LDL, low-density lipoprotein(s).

if thyroid hormones influence acid cholesterol ester hydrolase activity in rat liver, heart, and epididymal fat pads.

Experimental procedures

Materials

Cholesterol [1-^{14}C]oleate (specific activity 50 mCi/mmol) was purchased from New England Nuclear Canada, Lachine, Quebec, and was purified by Florosil chromatography [13]; [4-^{14}C]cholesterol oleate (specific activity 54 mCi/mmol) was also purchased from New England Nuclear Canada and was used without further purification. Unlabelled cholesterol oleate, lecithin (egg yolk, type IX-E), Hepes, calf-thymus DNA (type 1), and bovine serum albumin (fraction V) were obtained from Sigma Chemical Co., St. Louis, MO. 4-Methylumbelliferyl-2-acetamido-2-desoxy-β-D-glucopyranoside was purchased from Boehringer Mannheim (Canada) Ltd., St. Laurent, Quebec; 7-hydroxy-4-methylcoumarin and 3,5-diaminobenzoic acid were obtained from Aldrich Chemical Co., Milwaukee, WN. L-3,3′,5-Triiodothyronine (A grade) was obtained from Calbiochem, La Jolla, CA.

Animals and hormone treatments

Male Sprague-Dawley rats weighing 100–120 g were surgically thyroidectomized by Simonsen Laboratories, Gilroy, CA, and delivered, along with control litter mates, to the vivarium at the University of Calgary. Control and thyroidectomized rats were maintained for 2 weeks on standard laboratory chow and water containing 5% calcium gluconate. All animals were exposed to a 12-h alternating light cycle (light from 07.00–19.00 h). One-half of the control and thyroidectomized rats were then injected subcutaneously with triiodothyronine (50 μg/100 g body weight, dissolved in alkaline saline) for 3 consecutive days. The remaining rats received subcutaneous injections of the alkaline saline solution. Animals were killed by decapitation 24 h after the last injection and a serum sample was collected. Livers, hearts, and epididymal fat pads were individually removed, frozen rapidly and stored at –80°C until use.

Enzyme preparations

Livers and hearts were homogenized in 10 vol. of isotonic sucrose buffer (0.25 M sucrose/1 mM EDTA/10 mM HEPES, pH 7.5) at 4°C for 30 s with a Polytron PT-10 homogenizer (rheostat setting of 7); epididymal fat pads were homogenized in 3 vol. of buffer. An aliquot of the respective homogenates was removed, frozen and stored at –80°C for subsequent DNA determinations. Cellular debris was removed by centrifugation for 20 min at 5000×g; 59, 71 and 89% of the total homogenate acid cholesterol ester hydrolase activity was recovered in this fraction (average of three preparations from liver, heart and fat pads removed from control rats, respectively). Enzyme activities could be determined more reliably with this low-speed supernatant fraction than with the whole homogenate. All enzyme preparations were stored at –80°C prior to assay.

Cholesterol oleate substrate preparation

The glycerol-stabilized dispersion of cholesterol oleate was prepared essentially as described by Severson and Fletcher [14]. Cholesterol [1-^{14}C]oleate (3 μmol in benzene; specific activity 30 mCi/mmol) and lecithin (22.5 mol in chloroform) were dried under N_2. A small aliquot (100 μl) of absolute ethanol was added to the tube and the mixture was vortexed. Anhydrous glycerol (5 ml) was added, and the cholesterol oleate was dispersed by homogenization for 10–15 s at room temperature with a Polytron PT-10 disintegrator. Dissolved air bubbles were removed by centrifugation and the clear dispersion was stored at room temperature or at 4°C. The addition of ethanol enabled homogenizing times to be decreased from 5 min [14] to 10–15 s; ethanol had no effect in the subsequent cholesterol ester hydrolase activity determinations. Substrate dispersions of [4-^{14}C]cholesterol oleate in glycerol were prepared as described above.

Enzyme assays

Cholesterol ester hydrolase activity was determined as described previously by Severson and Fletcher [14]. Briefly, the glycerol-dispersed cholesterol oleate substrate was diluted with 5 vol. of buffer of an appropriate pH (0.1 M sodium acetate/1% w/v bovine albumin, pH 4–6; or 0.1 M sodium phosphate/1% w/v bovine albumin, pH 6–8), and 0.1 ml of this substrate mixture was incubated with appropriate quantities of enzyme protein for 30 min at

30°C in a total volume of 0.2 ml. Each assay contained 10 nmol cholesterol oleate (approximately 600 000 dpm) and 75 nmol of lecithin. Assay blanks contained buffer or water in place of the enzyme protein, and averaged 0.05% of the total substrate radioactivity. All assays were performed in duplicate. Incubations were terminated by the addition of 3.0 ml of a fatty acid extraction solution [15] and 0.5 ml of 0.5 M NaOH. The solution was vortexed, centrifuged and an aliquot (0.5 ml) of the upper phase (total volume of 2.0 ml) containing the sodium [1-^{14}C]-oleate was transferred to a scintillation minivial with 0.05 ml of 1 M HCl and 4 ml scintillation fluid. Radioactivity was measured in a Searle Mark III liquid scintillation spectrometer. The partitioning of free fatty acid (oleate) was monitored by the addition of known amounts of sodium [1-^{14}C]oleate to blank assay tubes; 90% was routinely recovered in the upper phase. A unit of cholesterol ester hydrolase was arbitrarily defined as that amount of enzyme which hydrolyzed 1 nmol of cholesterol oleate in one hour at 30°C; results from cholesterol ester hydrolase assays were calculated as units per mg 5000 $\times g$ supernatant protein or as units per mg homogenate DNA.

Assays with [4-^{14}C]cholesterol oleate as substrate were performed as outlined above, except that the reaction was terminated by the addition of 3 ml of chloroform/methanol (2 : 1). After the addition of 1 ml of H_2O, the assay tubes were vortexed and centrifuged. A 1-ml aliquot of the lower phase (total volume of 2.2 ml) was dried under N_2, redissolved in a small volume of chloroform/methanol (2 : 1, containing 1 μmol of cholesterol) and applied to a glass silica gel thin-layer plate. The thin-layer chromatographic separation procedure employed a solvent system consisting of hexane/diethyl ether/glacial acetic acid (73 : 25 : 2). The cholesterol-containing area of the plate was visualized under iodine vapor, scraped into glass scintillation vials containing 10 ml of scintillation fluid, and the radioactivity was measured.

The lysosomal marker enzyme *N*-acetylglucosaminidase was assayed by the fluorometric method as described by Peters et al. [16]; a unit of enzyme activity was arbitrarily defined as that amount of enzyme which hydrolyzed 1 μmol of substrate per h at 37°C. Under the experimental conditions of this investigation which used frozen rat tissues that were vigorously homogenized, no latency could be detected for the lysosomal marker enzyme and so assays were routinely determined in the absence of Triton X-100.

Other assays

DNA was measured in aliquots of tissue homogenates by the fluorometric method of Thomas and Farquhar [17] using calf thymus DNA as standard. Protein was measured by the fluorometric technique of Robrish et al. [18] using bovine albumin as the protein standard. Serum TSH levels were measured by radioimmunoassay by the Department of Laboratories, Foothills Hospital, Calgary, Alberta, using human TSH as the standard.

Results

Acid cholesterol ester hydrolase activity in rat liver, heart and epididymal fat pads

Cholesterol ester hydrolase activity, measured at pH 5, was linear with respect to time with low-speed supernatant preparations from liver, heart and fat pads removed from control rats (Fig. 1). The effect of pH on cholesterol ester hydrolase activity is shown in

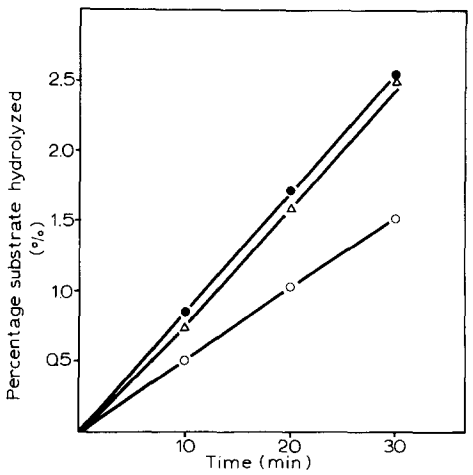

Fig. 1. Assay time course for acid cholesterol ester hydrolase activity in liver, heart and fat pad preparations from control rats. Cholesterol ester hydrolase activity, expressed as the percentage of the total substrate hydrolyzed, was determined at the indicated times of incubation with low-speed supernatant fractions from: liver (○, 63 μg protein), heart (△, 83 μg protein) and epididymal fat pads (●, 2 μg protein).

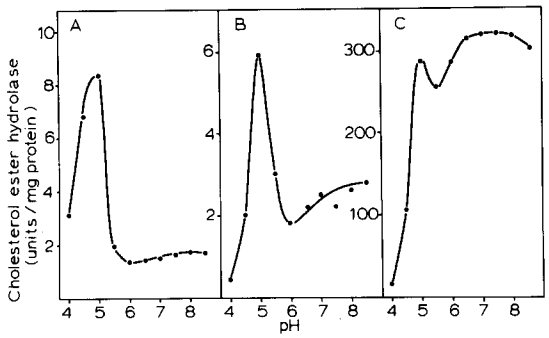

Fig. 2. Effect of pH on acid cholesterol ester hydrolase activity. Hydrolase activity was measured at the indicated pH values with the following low-speed supernatant preparations from control rats: panel A, liver (136 μg protein); panel B, heart (83 μg protein); panel C, fat pad (2 μg protein).

Fig. 2. Hydrolase activity with an acid pH optimum (pH 5) was clearly observed with preparations from liver (panel A) and heart (panel B). Acid cholesterol ester hydrolase activity with a pH optimum at pH 5 could also be observed in fat pads (panel C), although the considerable hydrolase activity at neutral pH values made the pH profile of the acid hydrolase less distinct than was observed for liver and heart (Fig. 2). It should be noted that the specific enzyme activity for the fat pad cholesterol ester hydrolase is much greater than the specific activity for the liver and heart hydrolase. The percentage distribution of acid cholesterol ester hydrolase activity (measured at pH 5) in particulate and soluble subcellular fractions obtained by differential centrifugation was very similar with that of lysosomal marker enzymes (*N*-acetylglucosaminidase and β-glucuronidase, results not shown), suggesting that the acid hydrolase in rat liver, heart and fat pads is of lysosomal origin. These results are in agreement with other investigations on acid cholesterol ester hydrolase in rat liver [19,20].

The validity of using a cholesterol ester substrate with the radioactive label in the fatty acid position for a cholesterol ester hydrolase assay was evaluated by comparing enzyme activity from rat liver with cholesterol [1-^{14}C]oleate and [4-^{14}C]cholesterol oleate substrate emulsions. Hydrolase activity determined at pH 5 with a rat liver 5000 × *g* supernatant preparation incubated with cholesterol [1-^{14}C]oleate and [4-^{14}C]cholesterol oleate substrate emulsions was 21.0 and 16.4 units/mg protein, respectively. Since these values are within the normal variation between individual substrate preparations, it was concluded that enzyme assays performed with a cholesterol [1-^{14}C]oleate substrate give a valid measurement of cholesterol ester hydrolase activity.

Effect of thyroid hormones on acid cholesterol ester hydrolase activity in rat liver, heart and epididymal fat pads

The effect of thyroidectomy and administration of thyroid hormones on the body weight of the experimental rats are shown in Table I. The thyroidectomized animals (group A) weighed significantly less than the age-matched euthyroid controls (group B). Administration of triiodothyronine to the thyroidectomized animals (group C) for 3 days resulted in no significant change in body weight; in contrast, 3 days of triiodothyronine treatment of control rats (group D) produced an average net weight loss of 9 g in comparison to an average net weight gain of 21 g for euthyroid controls (group B) in the same time interval. The effectiveness of the surgical thyroidectomy and the effects of administration of thyroid hormone was monitored at the time of killing by determining serum TSH levels (Table I). Serum TSH levels were significantly elevated in thyroidectomized animals in comparison to euthyroid control rats; triiodothyronine-treatment produced a significant decrease in serum TSH levels for both thyroidectomized and control animals.

TABLE I

EFFECT OF THYROIDECTOMY AND THE ADMINISTRATION OF TRIIODOTHYRONINE ON BODY WEIGHT AND SERUM TSH LEVELS OF RATS

Results are expressed as mean ± S.E.. The number of animals in each case was 9

Experimental group	Body weight (g)	Serum TSH (μU/ml)
A. Thyroidectomized	148 ± 7	8.5 ± 0.2
B. Control	274 ± 5	3.5 ± 0.2
C. Thyroidectomized plus triidothyronine	156 ± 6	2.4 ± 0.1
D. Control plus triiodothyronine	258 ± 2	2.2 ± 0.1

Since thyroidectomy resulted in a change in body weight (Table I) and in the wet weights of the livers, hearts and fat pads, all results for acid cholesterol ester hydrolase activity are expressed as both units per mg 5000 × *g* supernatant protein and as units per mg homogenate DNA. The effect of thyroidectomy and the administration of triiodothyronine on liver acid cholesterol ester hydrolase activity is shown in Table II. Thyroidectomy resulted in a decrease in liver wet weight (4.2 ± 0.3 g; mean ±S.E., $n = 9$) and liver weight as a percentage of body weight (2.9 ± 0.2%) in comparison to the euthyroid control (11.4 ± 0.6 g; 4.2 ± 0.2%). Thyroidectomy resulted in a significant decrease (25–35%) in liver acid cholesterol ester hydrolase activity; liver 5000 × *g* supernatant preparations from both control and thyroidectomized rats exhibited the same distinct acid pH optimum of 5.0 (results not shown). Administration of thyroid hormone (triiodothyronine) produced an increase in acid cholesterol ester hydrolase activity of approximately 3-fold and 1.5-fold for both thyroidectomized and euthyroid control animals, respectively (Table II). Thus, the effects of thyroid hormones and thyroidectomy on liver acid cholesterol ester hydrolase activity are identical with those effects reported for acid lipase activity in rat liver [3,4] and for lysosomal proteases in liver and skeletal muscle [1,2]. The specificity of the response of the liver to thyroid hormone was determined by measuring *N*-acetylglucosaminidase activity, a lysosomal marker enzyme. Although thyroidectomy did not change the activity of *N*-acetylglucosaminidase, administration of triiodothyronine did result in a significant elevation of *N*-acetylglucosaminidase activity in both thyroidectomized and euthyroid groups (Table II); similar effects of thyroid hormones on NAGA activity have been reported for liver and skeletal muscle [2,3].

The experimental results for the effects of thyroidectomy and thyroid hormones on acid cholesterol ester hydrolase activity in rat heart 5000 × *g* supernatant preparations are shown in Table III. Once again, heart wet weight was significantly decreased in the hypothyroid (thyroidectomized) animals (0.41 ± 0.03 g; mean ±S.E.; $n = 9$) compared with hearts from

TABLE II

EFFECT OF THYROIDECTOMY AND THE ADMINISTRATION OF TRIIODOTHYRONINE ON CHOLESTEROL ESTER HYDROLASE AND *N*-ACETYLGLUCOSAMINIDASE ACTIVITY IN RAT LIVER PREPARATIONS

Results are mean ± S.E. for nine preparations. *P* values were calculated by Student's *t*-test; n.s., not significant

Experimental group	Acid cholesterol ester hydrolase activity		*N*-Acetylglucosaminidase activity (units/mg protein)
	Units/mg protein	Units/mg DNA	
A. Thyroidectomized	9.05 ± 0.43	259 ± 42	0.68 ± 0.03
B. Control	12.04 ± 1.01	403 ± 32	0.68 ± 0.04
C. Thyroidectomized plus triiodothyronine	31.40 ± 1.88	724 ± 56	1.13 ± 0.05
D. Control plus triiodothyronine	21.59 ± 1.03	558 ± 40	0.96 ± 0.03
B versus A	$P < 0.02$	$P < 0.02$	n.s.
C versus A	$P < 0.001$	$P < 0.001$	$P < 0.001$
D versus B	$P < 0.001$	$P < 0.01$	$P < 0.001$

TABLE III

EFFECT OF THYROIDECTOMY AND THE ADMINISTRATION OF TRIIODOTHYRONINE ON CHOLESTEROL ESTER HYDROLASE AND *N*-ACETYLGLUCOSAMINIDASE ACTIVITY IN RAT HEART PREPARATIONS

Results are mean ± S.E. for nine preparations. *P* values were calculated by Student's *t*-test; n.s., not significant

Experimental group	Acid cholesterol ester hydrolase activity		*N*-Acetylglucosaminidase activity (units/mg protein)
	Units/mg protein	Units/mg DNA	
A. Thyroidectomized	5.91 ± 0.73	170 ± 27	0.12 ± 0.01
B. Control	4.99 ± 0.16	169 ± 11	0.14 ± 0.01
C. Thyroidectomized plus triiodothyronine	5.28 ± 0.33	213 ± 15	0.13 ± 0.01
D. Control plus triiodothyronine	4.41 ± 0.18	196 ± 7	0.14 ± 0.01
B versus A	n.s.	n.s.	$P < 0.05$
C versus A	n.s.	n.s.	n.s.
D versus B	$P < 0.05$	n.s.	n.s.

TABLE IV

EFFECT OF THYROIDECTOMY AND ADMINISTRATION OF TRIIODOTHYRONINE ON CHOLESTEROL ESTER HYDROLASE AND *N*-ACETYLGLUCOSAMINIDASE ACTIVITY IN RAT EPIDIDYMAL FAT PAD PREPARATIONS

Results are the mean ± S.E. for nine preparations. *P* values were calculated by Student's *t*-test; n.s., not significant

Experimental group	Acid cholesterol ester hydrolase activity		*N*-Acetylglucosaminidase activity (units/mg protein)
	Units/mg protein	Units/mg DNA	
A. Thyroidectomized	121 ± 14	1520 ± 190	0.24 ± 0.03
B. Control	204 ± 7	2220 ± 85	0.27 ± 0.02
C. Thyroidectomized plus triiodothyronine	149 ± 14	2760 ± 246	0.17 ± 0.01
D. Control plus triiodothyronine	227 ± 9	2920 ± 145	0.28 ± 0.02
B versus A	$P < 0.001$	$P < 0.01$	n.s.
C versus A	n.s.	$P < 0.01$	n.s.
D versus B	n.s.	$P < 0.001$	n.s.

euthyroid control rats (0.90 ± 0.03 g). Administration of triiodothyronine increased the average wet weight of the hearts from thyroidectomized and control rats to 0.61 ± 0.03 g and 1.21 ± 0.02 g, respectively. However, thyroidectomy or treatment with triiodothyronine produced no significant or consistent alteration in acid cholesterol ester hydrolase activity or in *N*-acetylglucosaminidase activity.

The effects of thyroidectomy and the administration of thyroid hormones on cholesterol ester hydrolase activities in the low-speed supernatant fraction from epididymal fat pad homogenates are shown in Table IV. As noted previously for wet weights of livers and hearts, the wet weight of the pair of epididymal fat pads (0.53 ± 0.06 g; mean ±S.E.; $n = 9$) from thyroidectomized animals was significantly less than the wet weight of fat pads removed from euthyroid controls (1.48 ± 0.07 g). Thyroidectomy results in a decrease in acid cholesterol ester hydrolase activity in comparison to the euthyroid controls. Administration of triiodothyronine to thyroidectomized and control rats produced an increase in acid cholesterol ester hydrolase activity; however, this effect was statistically significant only when results were expressed as units per mg DNA. Thus, the regulation of acid cholesterol ester hydrolase by thyroid hormones in adipose tissue (Table IV) is similar to that observed for the regulation of the rat liver enzyme (Table II), although the absolute magnitude of the effect of triiodothyronine on acid cholesterol ester hydrolase activity in fat pads from thyroidectomized rats (1.8-fold), for example, is less than the effect in liver (2.8-fold). In contrast to results with the liver (Table II), thyroid hormones did not significantly change *N*-acetylglucosaminidase activity in fat pad 5000 × *g* supernatant preparations (Table IV).

Discussion

This investigation has employed glycerol-dispersed cholesterol oleate for the characterization of cholesterol ester hydrolase activity in several rat tissue preparations. Characterization of acid cholesterol ester hydrolase activity in rabbit aorta with this glycerol-dispersed substrate [14] has revealed properties that were very similar to those observed with micellar [14] or phospholipid-vesicular (21) substrate preparations. The low-speed supernatant fraction from rat liver was observed to have predominantly an acid pH optimum of 5; similar results have been reported by other investigators for rat, human and bovine liver [19,20,22–24]. An acid cholesterol ester hydrolase was also observed in rat heart; previous studies from this laboratory have reported that particulate fractions from rat heart homogenates have triacylglycerol hydrolase (lipase) activity with an acid pH optimum of 4.5–5 [25]. In the case of the fat pad, acid hydrolase activity was partially obscured by the presence of substantial cholesterol ester hydrolase activity at neutral pH values, but a distinct pH optimum of 5 was still observed. Previous studies on cholesterol ester hydrolases from rat adipose tissue have primarily focussed on the activation of enzyme activity at neutral pH by a cyclic AMP-dependent protein kinase-catalyzed phosphorylation [26]. Riddle et al. [27] have reported that rat adipose tissue had some activity at acid pH values but much greater activity at neutral pH such that only a single pH optimum of about 7 was observed. The absence of a distinct acid

pH optimum in the investigation by Riddle et al. [27] may be due to their use of an acetone-dispersed cholesterol oleate substrate preparation. Severson and Fletcher [14] and Brecher et al. [21] have both reported that ethanol- or acetone-dispersed cholesterol oleate substrates were not hydrolyzed at acid pH values with rabbit aorta (media) preparations which were characterized as having an acid pH optimum with other substrate preparations. An acid cholesterol ester hydrolase (pH optimum of 4.5–5) has also been reported in enzyme preparations from chicken [14] and pigeon [28] adipose tissue in studies with the glycerol-dispersed cholesterol oleate substrate preparation. Since the distribution of the acid cholesterol ester hydrolase in rat liver, heart and fat pad preparations was similar to that of *N*-acetylglucosaminidase and β-glucuronidase, it is reasonable to conclude that the acid hydrolase is of lysosomal origin.

The liver plays an important role in the catabolism of lipoproteins. Since the degradation of cholesterol ester-labelled chylomicron remnant particles by rat hepatocyte monolayers was inhibited by chloroquine, a lysosomotropic agent, an acid lysosomal cholesterol ester hydrolase has been implicated in the lysosomal hydrolysis of the internalized chylomicron remnant [5,6] in the liver. In addition, recent studies with sucrose-derivatized LDL in swine have demonstrated that liver lysosomes accounted for about 40% of the LDL catabolized in 24 h [7]. The cholesterol ester component of LDL can also be degraded by lysosomal hydrolases at extra-hepatic tissue sites [8,9], and Pittman et al. [7] have shown that adipose tissue represents one of the major sites for the lysosomal catabolism of LDL. This finding is consistent with the observations of Angel et al. [29] concerning the binding, internalization and degradation of LDL with human fat cells; a high specific ^{125}I-labelled LDL binding activity has also been reported for bovine adipose tissue membrane fragments [30]. A recent communication has shown that neutral cholesterol ester hydrolases in rat and chicken adipose tissue will hydrolyze the cholesterol esters in rat lipoprotein fractions [31]. However, the contribution of adipose tissue lysosomes to the degradation of LDL in vivo has been clearly demonstrated with the use of sucrose-derivatized LDL [7], and the presence of fat pad acid cholesterol ester hydrolase activity as shown in the present investigation is consistent with this lysosomal mechanism. Although an acid cholesterol ester hydrolase was also observed in rat heart, this presumably has little or no physiological role to play in terms of lipoprotein degradation since the uptake of chylomicron cholesterol esters has been described in perfused hearts as a non-saturable, receptor-independent process that does not involve endocytosis [32].

Hypercholesterolemia is a common feature in humans who are hypothyroid, and the administration of thyroid hormones to these hypothyroid individuals results in a rapid decrease in serum cholesterol levels [9–11]. The mechanism by which thyroid hormones reduce serum cholesterol levels has been reported to be due to an increase in the hepatic catabolism of cholesterol [33]. The present study has shown that thyroidectomy resulted in a decrease in liver acid cholesterol ester hydrolase activity relative to the euthyroid controls, indicating that physiological levels of thyroid hormones regulate lysosomal acid cholesterol ester hydrolase activity. Administration of thyroid hormone (triiodothyronine) in pharmacological doses for 3 days produced an increase in acid cholesterol ester hydrolase activity in livers from both thyroidectomized and euthyroid control rats. Thus, regulation of lysosomal acid cholesterol ester hydrolase activity in the liver by thyroid hormones may contribute to the effect of thyroid hormones on hepatic cholesterol catabolism. The present results showing the increase in liver acid cholesterol ester hydrolase activity due to thyroid hormones confirm the earlier observations by Coates et al. [3,4] on the effect of thyroid hormones on liver acid lipase activity. In terms of hepatic cholesterol ester metabolism, the present observations regarding the regulation of liver acid cholesterol ester hydrolase activity by thyroid hormones were necessary since recent studies by Koster et al. [34] have suggested that the acid lipase (measured with 4-methylumbelliferyl oleate) and acid cholesterol ester hydrolase may be different enzymes.

Hypothyroidism in rats has been reported to result in a decrease in the fractional catabolic rate of ^{125}I-labelled LDL [35], and therefore thyroid hormones could influence LDL catabolism at extra-hepatic sites as well. Recently, Chait et al. [36] have reported that exposure of cultured human fibroblasts to physiologi-

cal concentrations of triiodothyronine increased the binding and degradation of LDL. Thyroidectomy produced a significant decrease in acid cholesterol ester hydrolase activity in fat pad preparations, and treatment with triiodothyronine resulted in an increase in acid enzyme activity in fat pad preparations from both euthyroid control and thyroidectomized rats which was significant when results were expressed as units of activity per mg DNA. Once again, the consistent effects of thyroidectomy to decrease acid cholesterol ester hydrolase activity suggest that acid hydrolase activity in fat pads can be regulated by physiological levels of thyroid hormones. Thyroidectomy and the administration of triiodothyronine produced no change in *N*-acetylglucosaminidase activity in fat pad preparations; other investigators have also noted that effects of triiodothyronine on rat liver acid lipase activity can be dissociated from changes in *N*-acetylglucosaminidase activity [4], indicating some differences can exist with respect to the sensitivity of various lysosomal hydrolases towards regulation by thyroid hormones.

These results suggest that the effects of hypothyroidism on the fractional catabolic rate of LDL could be due, at least in part, to alterations in acid cholesterol ester hydrolase activity in both liver and adipose tissue. In this regard, it is interesting to note that thyroid hormones had no effect on acid cholesterol ester hydrolase in heart where no lysosomal degradation of lipoproteins has been observed. This finding of some degree of tissue specificity towards the effects of thyroid hormones on acid hydrolase activity is consistent with the report of DeMartino and Goldberg [2] that thyroid hormones did not alter the activity of lysosomal proteases in tissues (heart and kidney) where thyroid hormones did not influence rates of protein degradation. The suggestion that the regulation of acid cholesterol ester hydrolase activity in liver and adipose tissue by thyroid hormones may be relevant with respect to determining physiological rates of lipoprotein degradation is subject to two important qualifications. First, it must be assumed that the glycerol-dispersed cholesterol oleate substrate dispersion gives a valid measure of the activity of lysosomal acid hydrolases. Second, at least in the case of hypothyroidism, it must be assumed that the activity of lysosomal acid hydrolases will be a rate-limiting step in lipoprotein degradation.

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