Secretion and Degradation of Lipoprotein Lipase in Cultured Adipocytes

BINDING OF LIPOPROTEIN LIPASE TO MEMBRANE HEPARAN SULFATE PROTEOGLYCANS IS NECESSARY FOR DEGRADATION*

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Equilibrium-binding data of highly purified 125I-labeled avian lipoprotein lipase to cultured avian adipocytes demonstrate the presence of a class of high affinity binding sites. Analysis of the binding function yielded an association constant of $0.62 \times 10^8 \text{ M}^{-1}$ and a maximum binding capacity of 2.1 $\mu$g/60-mm dish. From a time course of dissociation of 125I-lipoprotein lipase from adipocytes at 4°C, a dissociation rate constant of $6.1 \times 10^{-9} \text{ s}^{-1}$ was obtained. Pretreatment of cells with heparinase and heparitinase resulted in a quantitative suppression of the high affinity binding component, establishing that lipoprotein lipase is bound to cell surface heparan sulfate proteoglycans. At 37°C, cell surface-bound 125I-lipoprotein lipase is internalized and either degraded or recycled to the medium. The degradation rate constant for 125I-lipoprotein lipase was estimated to be 0.78 h$^{-1}$. The degradation rate constant was reduced 6-fold when cells were exposed to 100 $\mu$M chloroquine, indicating that most of the degradation occurs within the lysosomal compartment. By using cells that had been pulsed with Trans35S-S" for 1 h, it was demonstrated that acute treatment with endoglycosidases for up to 1 h resulted in a new lipoprotein lipase secretion rate which was 6-fold higher than that of control cells. Degradation of newly synthesized lipoprotein lipase was essentially blocked 30 min after the initiation of the chase. In other studies it was observed that there were no additive effects of chloroquine and either endoglycosidase or heparin treatment on total lipoprotein lipase levels (intracellular, cell surface, and medium) in adipocyte cultures. These experiments support the hypothesis that the release of lipoprotein lipase from its receptor prevents its internalization and degradation and enhances enzyme efflux from the adipocyte. A new model of lipoprotein lipase secretion in cultured adipocytes is proposed: Newly synthesized lipoprotein lipase is transported to the cell surface where it binds to specific heparan sulfate proteoglycan receptors. The enzyme is either released to the medium or internalized via the receptor, in which case the enzyme is degraded or recycled to the cell surface. Major determinants of enzyme efflux from the cell surface include the number and integrity of receptors, the association constant of the enzyme-receptor complex, and the presence in the medium of competing molecules with high affinity for lipoprotein lipase. In this model, modulation of lipoprotein lipase degradation rate may be a significant mechanism for acute regulation of enzyme efflux independent of changes in the rate of enzyme synthesis.

Lipoprotein lipase is the major enzyme responsible for the hydrolysis of VLDL and chylomicron triglycerides (1, 2). In adipose tissue, lipoprotein lipase is synthesized and secreted by adipocytes and transported to the surface of endothelial cells, where it is believed to function in vivo (2). Heparan sulfate proteoglycans provide high affinity lipoprotein lipase binding sites on the endothelium (3, 4). The mechanism of lipase transport from adipocytes to endothelial cells, however, has not been elucidated.

Known effectors of lipoprotein lipase protein and activity include insulin (5-7), CAMP (8, 9), and heparin (10, 11). These effectors act through a variety of mechanisms at the molecular level. Insulin has been shown to increase lipoprotein lipase synthesis as part of an increase in total protein synthesis in rat adipose tissue cultures (5) and in cultured 3T3-L1 adipocytes (6). In addition, a specific effect of insulin on lipoprotein lipase synthesis may be mediated by glucocorticoids (6). Researchers have also proposed that insulin affects both intracellular and intercellular lipoprotein lipase transport (6, 7). In contrast, CAMP-mediated decreases in lipoprotein lipase activity have been attributed to decreased lipoprotein lipase synthesis and increased lipoprotein lipase catalytic efficiency (9). Recent studies with cultured avian adipocytes show that heparin decreases the degradation rate and half-intracellular residence time (10) of lipoprotein lipase. In control adipocyte cultures, 76% of the newly synthesized enzyme was degraded. Addition of heparin to culture media reduced the degradation rate so that only 21% of the newly synthesized enzyme was degraded. These studies raised the possibility that changes in degradation rate might be a mechanism for rapidly altering enzyme secretion rate independently of changes in synthesis.

The objective of the present study was to determine the mechanism underlying the turnover of lipoprotein lipase in cultured adipocytes. We hypothesize that in control cells newly synthesized lipoprotein lipase is transported to the plasma membrane where it binds to heparan sulfate proteoglycans. The release of enzyme to the medium is governed by the dissociation constant of the enzyme-proteoglycan complex

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1 The abbreviations used are: VLDL, very low density lipoprotein; PBS, phosphate-buffered saline; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate; ELISA, enzyme-linked immunosorbent assay.
and the residence time of the proteoglycan receptor on the cell surface. The majority of enzyme molecules are internalized, and either degraded in the lysosomal compartment or recycled to the cell surface. In this report we have provided evidence for specific binding of lipoprotein lipase to the adipocyte cell surface in culture and for internalization, degradation, and partial recycling to the medium of exogenous $^{125}$I-labeled enzyme. In addition, we have shown that manipulations which prevent binding of lipoprotein lipase to cell surface proteoglycans, such as treatment with heparinase and heparin, decrease the degradation rate of lipoprotein lipase and enhance the rate of enzyme efflux from adipocytes to the culture medium.

**EXPERIMENTAL PROCEDURES**

**Materials**—Heparinase from either ICN Immunobiologicals or Sigma was used in this study. Heparinase was obtained from ICN Immunobiologicals. Trans-$^{3}$H-label and $^{125}$I-label were obtained from ICN Radiochemicals. In this manuscript, 1 unit of either heparinase or heparin is defined as approximately 0.066 IU as currently defined by ICN. Bovine serum albumin (A-7030), essentially fatty acid-free, was obtained from Sigma.

**Preparation of Avian Adipocytes, Cell Culture, and Sample Collection**—Adipocytes were prepared as described by Cupp et al. (10) and plated in 60-mm culture dishes. By day 3 or 4 in culture, either 80% or all of the medium was removed and replaced with an equal volume of RPMI 1640 containing either 0.5 or 1% hepatic serum and 5.6 mM glucose, 1.3 mM L-glutamine, 50 mM/ml L-glutamine, 50 mM/ml streptomycin, 100 mM/ml neomycin, 10 mM HEPES, and 10 mM/ml insulin (complete RPMI 1640) and supplemented with 20 μM deamethasone and 0.5% methylcellulose. Eighty percent of the medium was replenished every third day thereafter except in the case of equilibrium-binding studies, when media were changed 1-3 h before the addition of $^{125}$I-lipoprotein lipase.

In experiments designed to measure the effect of the endoglycosidases on lipoprotein lipase, cells were rinsed with 2 ml of 0.15 M NaCl, 5 mM phosphate, pH 7.4 (PBS), and treated as described in figure and table legends. Following experimental manipulations, media were collected, cells were rinsed with 2 ml of ice-cold PBS, and 1 ml of ice-cold complete RPMI 1640 (without hepatic serum) supplemented with 10 units/ml heparin was added to each dish. After a 10-min incubation on ice with shaking, the heparin wash was collected. This wash contained cell surface-associated enzyme. Finally, cells were rinsed with 2 ml of ice-cold PBS and harvested in 0.5 ml/dish of lysis buffer B (0.75 M NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 10 units/ml heparin, and 10 mM HEPES, pH 7.4) or lysis buffer A (4 mM CHAPS, 5 units/ml heparin, and 50 mM NH$_4$OH, pH 8.1) for experiments with or without isotypes, respectively. Both lysis buffers contained antiproteolytic agents (1 μg/ml leupeptin, 2 μg/ml antipain, 10 μg/ml benzamidine, 10 units/ml Trasylol, 1 μg/ml chymostatin, and 10 μg/ml pepstatin). Cell extracts were sonicated at 50 watts for 15 s using a Braun-sonic 1510 probe sonicator equipped with a microprobe (4 mm). Extracts were prepared for immunoadsorption by centrifugation at 30,000 rpm in a Beckman 40.5 rotor for 45 min at 4°C. Cellular debris was removed from media and heparin washes by centrifugation at 1000 $\times$ g for 30 min at 4°C. Lipoprotein lipase protein or radioactivity in media, heparin washes, and cell extracts was measured by ELISA or immunoadsorption, respectively.

**Studies with $^{125}$I-Lipoprotein Lipase**—Highly purified avian adipose lipoprotein lipase (155 μg/100 μl) (13) was iodinated with 1 μCi of $^{125}$I using lactoperoxidase coupled to Sepharose 4B (12, 13). To separate $^{125}$I-lipoprotein lipase from high molecular weight material, which was loaded on a heparin-Sepharose 4B column (0.5 x 6.5 cm) that had been equilibrated with 0.15 M NaCl, 30% glycerol, 10 mM phosphate buffer, pH 7.0. After the column was washed with at least 4 bed volumes of equilibration buffer, $^{125}$I-lipoprotein lipase was eluted with 1.5 ml of 0.15 M NaCl, 5% glycerol, 10 mM phosphate buffer, pH 7.0. The labeled protein was stored at −20°C.

For equilibrium-binding studies, media were changed 1–3 h prior to the initiation of experiments to release cell surface lipoprotein lipase (10). Replacement medium consisted of complete RPMI 1640 supplemented with 0.2% bovine serum albumin. Following the incubation at 37°C, cells were placed on ice and rinsed with 2 ml of ice-cold PBS. Fresh medium containing 0.2% bovine serum albumin was added to dishes, followed by the addition of $^{125}$I-lipoprotein lipase. Cells were then incubated at 4°C for 2 h with shaking. Media were removed, and 2 ml of ice-cold PBS or 4 x 2 ml of ice-cold medium containing 0.2% bovine serum albumin. Bound $^{125}$I-lipoprotein lipase was released in 2 x 1-ml heparin washes. For these experiments, the heparin wash medium was supplemented with 0.2% bovine serum albumin. $^{125}$I-Lipoprotein lipase was precipitated from media and heparin washes with 10% trichloroacetic acid. The precipitate was pelleted by centrifugation at 15,000 g at 4°C in Beckman microcentrifuge tubes treated with 0.1% trichloroacetic acid, and counted in a Beckman Biogamma counter.

To determine the dissociation rate constant and the half-life of dissociation of lipoprotein lipase bound to receptors in adipocyte cultures, cells were incubated with 10 μg of $^{125}$I-lipoprotein lipase for 2 h at 4°C on ice with shaking. Following 2 x 2-ml rinses with ice-cold PBS, medium containing 10 μg of unlabeled lipoprotein lipase was added to dishes. Cells were then incubated at 4°C on ice with shaking for up to 5 h. $^{125}$I-Lipoprotein lipase was measured in media, heparin washes, and cell extracts following precipitation by trichloroacetic acid.

Internalization and degradation of $^{125}$I-lipoprotein lipase was measured in cultures that had been incubated with 0.38 μg of $^{125}$I-lipoprotein lipase for 30 min at 4°C. The medium had been removed the day before and replaced with complete RPMI 1640. At the medium change, the day before experiments in experiments with ionophores and chloroquine, cells were preincubated with 100 μg/ml of 2 (155 pg/150 ml) $^{125}$I-lipoprotein lipase in media, heparin washes, and cell extracts was measured following precipitation by trichloroacetic acid. Degradation of $^{125}$I-lipoprotein lipase was determined by measurement of $^{125}$I-tyrosine as described by Bierman et al. (14). From the rate of release of $^{125}$I-tyrosine, it was determined that by 30 min at 37°C there was a maximal accumulation of intracellular $^{125}$I-lipoprotein lipase. To examine the fate of internalized lipoprotein lipase, cultures that had been incubated with $^{125}$I-lipoprotein lipase for 30 min at 4°C were then incubated for 30 min at 37°C to allow for internalization of bound $^{125}$I-lipoprotein lipase. Following this incubation, the medium was removed, cells were rinsed with 2 ml of ice-cold PBS, and 2 ml of diluted spent medium were added to dishes. Cultures were then incubated at 37°C for up to 2 h. $^{125}$I-Lipoprotein lipase in media, heparin washes, and cell extracts was measured following precipitation by trichloroacetic acid. Bound $^{125}$I-lipoprotein lipase was released with 2 x 1-ml heparin washes. Cultures were rinsed with 5 x 2 ml of cold PBS, and 2 ml of diluted spent media were added to dishes. Cultures were then incubated at 37°C for 1 h. Media containing intracellular $^{125}$I-lipoprotein lipase was collected. $^{125}$I-Lipoprotein lipase and $^{125}$I-tyrosine were measured as described. For experiments with chloroquine, fresh media, rather than diluted spent media, were added to dishes for 37°C incubations.

**Measurement of Lipoprotein Lipase**—Lipoprotein lipase protein was measured by ELISA. Assay conditions were similar to those described for hepatic lipase (15, 16) with the following changes. Microtiter plates were coated overnight with 1 μg of affinity-purified immunoglobulin in 200 μl of coating buffer; sodium dodecyl sulfate was omitted from the sample incubation; and the conjugate was diluted in 0.15 M NaCl, 1% bovine serum albumin, 0.05% Tween 20, 10 mM phosphate, pH 7.4. Immunoglobulins were affinity-purified from goat antisera against avian lipoprotein lipase as previously described (9, 13). Aliquots of a single pool of highly purified avian lipoprotein lipase (13), diluted in 1 M NaCl, 0.3% bovine serum albumin, 0.1% sodium azide, 0.5% methyl cellulose, 10 mM phosphate, pH 7.4, and stored at −70°C, were used for standards. The standard curve ranged from 0.03 to 1.5 ng of lipoprotein lipase. $^{35}$S-Labeled lipoprotein lipase was measured by immunoadsorption as described in detail by Cupp et al. (10). For synthesis experiments and the perturbation study, cultures were pulsed with the indicated times with 100 μCi/dish of Trans-$^{3}$H-label in complete RPMI 1640 containing only 5 μM methionine. In the perturbation study, cultures were chased with complete RPMI 1640 for up to 1 h.

**Other Methods**—For measurement of heparan sulfate proteoglycan, adipocytes were incubated for 48 h with 0.2% sodium dodecyl sulfate in complete RPMI 1640. Cells were rinsed with 2 ml of PBS and incubated with complete RPMI 1640 for 5 h at 37°C in the presence or absence of the endoglycosidases. Both heparinase and heparinase were present at a concentration of 0.5 units/ml. Medium,
trypsin-releasable, and cell-associated pools were collected and handled as described by Bienkowski and Conrad (17). DNA content was determined by nitrous acid degradation (18).

Total 1^35S-labeled protein was determined by the method of Miller and Carrino (19), using 0.2 mg of bovine serum albumin as carrier. Cellular protein was measured by the method of Lowry et al. (20). DNA content was determined using a fluorometric assay (21).

**RESULTS**

**Binding of 125I-Lipoprotein Lipase to Cultured Adipocytes—** A time course of binding of lipoprotein lipase to cultured adipocytes at 4 °C indicated that equilibrium was achieved by 1 h (data not shown). In all equilibrium-binding experiments, incubations were conducted for 2 h. It was verified in an experiment conducted at an enzyme concentration of 0.95 μg/ml that, at 4 °C, more than 95% of the cell-associated 125I-lipoprotein lipase was released by two heparin washes. Equilibrium-binding experiments yielded biphasic increases in cell surface-associated enzyme (Fig. 1). By assuming that total binding was the sum of a high affinity component and a non-specific contribution, the binding function can be described by

\[ \bar{V} = \frac{n_K[S]}{1 + K[S]} + a[S] \]

where \( \bar{V} \) represents the micrograms of lipoprotein lipase bound per dish; \([S]\), the concentration of free enzyme at equilibrium in micrograms/milliliter; \(K_0\), the association constant; \(n_1\), the maximum amount of enzyme specifically bound per dish in micrograms/dish; and \(a\), the slope of the non-specific binding function. Fitting the results of four binding experiments by the weighted least squares technique of Marquardt (22) yielded an association constant of 0.82 × 10^4 ± 0.16 × 10^4 M^−1 and maximum specific binding of 2.1 ± 0.4 μg/dish (mean ± S.D.). Competition of unlabeled lipoprotein lipase with 125I-lipoprotein lipase was conducted in a separate experiment. At an enzyme concentration of 0.19 μg/ml (specific activity 704,560 cpm/μg), 141,050 cpm/dish were bound. Addition of 29.8 μg/ml of nonradioactive lipoprotein lipase, a 157-fold excess, yielded binding of 25,200 cpm/dish or, taking into account the new specific activity, 5.6 μg/dish. This figure agrees with the predicted binding, 5.0 μg/dish, calculated from the binding function and utilizing the association constant and the maximum binding obtained with the same batch of cells. These data provide evidence that iodination of lipoprotein lipase did not affect its binding behavior. Pretreatment of adipocytes for 3 h with 0.5 units/ml of both heparinase and heparitinase reduced the binding of 125I-lipoprotein lipase dramatically (Fig. 1). In addition, the function describing bound 125I-lipoprotein lipase versus free lipase in the medium was linear with a slope, 0.036, similar to the slope of the non-specific component, 0.042, calculated for untreated cells. The dissociation rate constant for the lipoprotein-lipase-receptor complex was determined from a time course of dissociation of 125I-lipoprotein lipase from adipocytes at 4 °C (Fig. 2). A rate constant of 6.1 × 10^−3 s^−1 was obtained. This was equivalent to a half-life of 3.1 h. The above data demonstrate the presence of a class of high affinity binding sites for lipoprotein lipase on cultured adipocytes. In addition, the binding studies with endoglycosidase-treated cells indicate that lipoprotein lipase is bound to cell surface heparan sulfate proteoglycans or molecules resembling heparan sulfate.

**Effect of Treatment of Adipocytes with Heparinase and Heparitinase on Lipoprotein Lipase Secretion—** In order to test the hypothesis that binding of newly synthesized lipoprotein lipase to the plasma membrane led to partial internalization and decreased appearance of enzyme in the cell culture medium, the effect of stripping cell surface lipoprotein lipase receptors on enzyme secretion was measured. Adipocytes were cultured in the presence of either heparinase or heparitinase for 6 h at 37 °C. Fig. 3, A and B, shows that medium lipoprotein lipase increased relative to control values in both heparinase- and heparitinase-treated cultures, whereas cell surface and intracellular lipoprotein lipase decreased. Total lipoprotein lipase (sum of the intracellular, cell surface, and medium pools) increased by 67 and 42%, respectively. Maximal effects were observed at a concentration of 0.5 units/ml with heparinase treatment and at 0.2 units/ml with heparitinase treatment. Cell surface, medium, and total lipoprotein lipase levels in heparin-treated cultures were similar to those in heparinase-treated cultures (data not shown). Intracellular lipoprotein lipase decreased by 29% (data not shown) and 79%.

**Fig. 1.** Concentration-dependent binding of lipoprotein lipase (LPL) to adipocyte cultures. Adipocytes were maintained in complete RPMI 1640 containing 0.2% bovine serum albumin for 3 h at 37 °C in the presence or absence of both heparinase (0.5 units/ml) and heparitinase (0.5 units/ml). Cells were then incubated with increasing amounts of 125I-lipoprotein lipase (specific activity 5245 cpm/μg of protein) for 2 h at 4 °C. Incubation media were collected for measurement of free 125I-lipoprotein lipase. Bound 125I-lipoprotein lipase was released in 2 × 1 ml heparin washes. Binding curves from control (●) and endoglycosidase-treated (○) cultures are shown. Each point represents data from a single dish.

**Fig. 2.** Determination of the dissociation rate constant of lipoprotein lipase (LPL) from receptors in adipocyte cultures. Adipocytes were maintained in complete RPMI 1640 supplemented with 0.2% bovine serum albumin for 1.5 h prior to the addition of 5 μg/ml of 131I-lipoprotein lipase (specific activity 23,980 cpm/μg of protein). Cultures were incubated with the labeled enzyme for 2 h at 4 °C and washed with PBS, and fresh media containing 5 μg/ml of unlabeled lipoprotein lipase were added to dishes. Media and heparin washes were collected over the next 5 h as described under "Experimental Procedures." Total 131I-lipoprotein lipase (□), referring to the sum of surface-bound and medium enzyme, and bound 125I-lipoprotein lipase (△) are shown. Each point represents data from a single dish.
relative to controls, in heparin- and heparinase-treated cultures, respectively. This parallel between endoglycosidase and heparin treatments, however, was not quantitatively reproducible. In all experiments, medium lipoprotein lipase levels were increased relative to controls, but in most cases medium lipoprotein lipase levels were greater in heparin-treated cultures than in endoglycosidase-treated cultures. Fig. 3B shows that medium lipoprotein lipase increased 4.7-fold with heparitinase treatment, while in the same experiment medium lipoprotein lipase increased 8.4-fold with heparin treatment (data not shown).

To determine whether inactivation of the endoglycosidases could account for their inability to quantitatively mimic heparin, either heparinase or the combination of heparinase and heparitinase was added to adipocytes both at the initiation and halfway through a 5-h experiment. No differences were observed between lipoprotein lipase levels in cultures treated with either one or two additions of heparinase (data not shown), suggesting that enzyme activity of the endoglycosidases was maintained during experiments. Since treatment with both endoglycosidases increased medium lipoprotein lipase by 54% compared to treatment with heparinase alone, subsequent experiments were performed using the combination of both enzymes. The effectiveness of the endoglycosidase treatment was verified when cells that had been labeled with [35S]sulfate for 48 h were treated with the endoglycosidases. In this experiment, cell surface heparan sulfate proteoglycans decreased by 87% in endoglycosidase-treated cultures compared to controls (data not shown).

The differences between the effects of the endoglycosidases and heparin on lipoprotein lipase may also have been due to a lag time of endoglycosidase action. To test this possibility, cultures were treated with either the endoglycosidases or heparin for 3 h, media were changed, and the cultures were maintained for an additional hour. Medium lipoprotein lipase levels increased 35% in endoglycosidase-treated cultures, in contrast to 200% in heparin-treated cultures. These data suggest that heparin and the endoglycosidases exert their effects differently.

**Effect of Endoglycosidase Treatment on Lipoprotein Lipase Synthesis Rate**—To test whether the observed increases in medium lipoprotein lipase levels in endoglycosidase-treated cultures were due to increased enzyme synthesis, adipocytes were treated with both heparinase and heparitinase for the indicated times and pulsed with Trans35S-label" for either 5 or 15 min (Table I). Both the incorporation of 35S into lipoprotein lipase and the relative rate of lipoprotein lipase synthesis were 30–40% lower in endoglycosidase-treated cells than in control cells. Thus, endoglycosidase treatment actually decreased the rate of lipoprotein lipase synthesis in cultured adipocytes. Therefore, increases in enzyme protein accompanying endoglycosidase treatment must be a result of decreased lipoprotein lipase degradation.

In order to determine how rapidly the endoglycosidase could affect lipoprotein lipase distribution and turnover in adipocytes, cells were labeled with Trans35S-label" for 1 h and chased in the presence or absence of the endoglycosidases for up to 1 h. Fig. 4, B and C, shows that by 15 min there was 80% less labeled, cell surface lipoprotein lipase in treated cultures than in controls. In addition, there was 7-fold more labeled lipoprotein lipase in the medium of treated cultures than controls (Fig. 4, A and C). This increase could not be accounted for solely by the release of cell surface enzyme, but was also due to a decrease in intracellular lipoprotein lipase and perhaps a decrease in the degradation rate. From the measurement of total lipoprotein lipase in the medium, secretion rates of 9.6 and 1.5 ng/h/dish were calculated for endoglycosidase-treated and control cells, respectively. These enzyme mass data corroborate the immunoadsorption data obtained for secreted 35S-labeled lipoprotein lipase. Thus, one initial effect of endoglycosidase treatment is the increased delivery of lipoprotein lipase to the medium. Finally, by 1 h there was a decrease in the loss of total labeled lipoprotein.

**TABLE I**

Incorporation of Trans35S-label" into lipoprotein lipase

<table>
<thead>
<tr>
<th>Time of incorporation</th>
<th>Control</th>
<th>Heparinase + heparitinase treatment</th>
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<tbody>
<tr>
<td>min</td>
<td>cpm/8 dishes</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1715 ± 189</td>
<td>1223 ± 211*</td>
</tr>
<tr>
<td>5</td>
<td>160 ± 52</td>
<td>96.8 ± 5.8*</td>
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Relative rate of LPL synthesis

%  

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<tr>
<td>15</td>
<td>0.0033 ± 0.0003</td>
<td>0.0021 ± 0.0003*</td>
</tr>
<tr>
<td>5</td>
<td>0.0037 ± 0.0005</td>
<td>0.0022 ± 0.0003*</td>
</tr>
</tbody>
</table>

*p < 0.05.

*p < 0.10.
lipoprotein lipase (LPL) in cultured adipocytes. Adipocytes were pulsed with 100 μCi/dish of Trans-35S-label for 1 h and chased for up to 1 h in the presence or absence of both heparinase (0.5 units/ml) and heparitinase (0.5 units/ml). D shows total (sum of intracellular, cell surface, and medium) and heparitinase (0.5 units/ml) extracts at media, and heparin washes from pools of six dishes.

FIG. 4. The acute effects of endoglycosidase treatment on lipoprotein lipase (LPL) in cultured adipocytes. Adipocytes were incubated with fresh medium for up to 1 h. After the first 37 °C incubation, 65% of this lipoprotein lipase had been delivered to the medium.

FIG. 5. The degradation and recycling of 125I-lipoprotein lipase (125I-LPL) in adipocyte cultures. Adipocytes were incubated with 0.38 μg of 125I-lipoprotein lipase (specific activity 977,084 cpm/μg of protein) for 30 min at 4 °C, the medium was removed, and cells were incubated with fresh medium for 30 min at 37 °C. Cell surface lipoprotein lipase was then released in 2 × 1-ml washes at 4 °C on ice. Cells were rinsed with 5 × 2 ml of PBS and incubated with fresh medium for up to 60 min at 37 °C. Intracellular (O), cell surface (Δ), and medium (■) 125I-lipoprotein lipase were measured following precipitation with trichloroacetic acid. 125I-Tyrosine (□) was measured as described in the text. Each point represents data for a pool of two dishes.

The degradation and recycling of 125I-lipoprotein lipase (125I-LPL) in cultured adipocytes. Adipocytes were incubated at 37 °C for 30 min, washed with heparin-containing medium at 4 °C, and finally incubated at 37 °C for up to 1 h. After the first 37 °C incubation, 7.5% of the 125I-lipoprotein lipase that had been bound to the cell surface was inside the cell (Fig. 5). Following 1 h at 37 °C, 65% of this internalized lipoprotein lipase was degraded. In addition, at 1 h there was 3.5-fold more 125I-lipoprotein lipase in the medium than could be accounted for by release of enzyme from the cell surface, indicating that 16% of the internalized lipoprotein lipase had been delivered to the medium.

To investigate whether degradation of lipoprotein lipase occurs in lysosomes, the effects of the lysosomotropic agent chloroquine on lipoprotein lipase (LPL) in cultured adipocytes. Cells were incubated in the presence of chloroquine, as indicated, for 5 h at 37 °C. Lipoprotein lipase in cell extracts (O), media (■), and heparin washes (Δ) was measured by ELISA. Each point represents the mean ± S.D. from three pools of three dishes.

FIG. 6. The effects of chloroquine on lipoprotein lipase (LPL) in cultured adipocytes. Cells were incubated at 37 °C in the presence or absence of 100 μM chloroquine for 1 h prior to the addition of 0.70 μg of 125I-lipoprotein lipase (specific activity 409,459 cpm/μg of protein). Cultures were then handled as described in Fig. 5. Total 125I-lipoprotein lipase and total 125I-iodotyrosine, respectively, were measured in control (■, ■) and chloroquine-treated (O, O) cultures. Total lipoprotein lipase and total iodotyrosine refer to the sum of intracellular, cell surface, and medium measurements. Each point represents a pool of two dishes.

FIG. 7. The effects of chloroquine on internalized 125I-lipoprotein lipase (125I-LPL) in cultured adipocytes. Adipocytes were incubated at 37 °C for 30 min, washed with heparin-containing medium at 4 °C, and finally incubated at 37 °C for up to 1 h. After the first 37 °C incubation, 7.5% of the 125I-lipoprotein lipase that had been bound to the cell surface was inside the cell (Fig. 5). Following 1 h at 37 °C, 65% of this internalized lipoprotein lipase was degraded. In addition, at 1 h there was 3.5-fold more 125I-lipoprotein lipase in the medium than could be accounted for by release of enzyme from the cell surface, indicating that 16% of the internalized lipoprotein lipase had been delivered to the medium.

Internalization and Degradation of Exogenous Lipoprotein Lipase Bound to the Surface of Adipocytes—Following the binding of 125I-lipoprotein lipase to adipocytes at 4 °C, cultures were incubated at 37 °C for 30 min, washed with heparin-containing medium at 4 °C, and finally incubated at 37 °C for up to 1 h. After the first 37 °C incubation, 7.5% of the 125I-lipoprotein lipase that had been bound to the cell surface was inside the cell (Fig. 5). Following 1 h at 37 °C, 65% of this internalized lipoprotein lipase was degraded. In addition, at 1 h there was 3.5-fold more 125I-lipoprotein lipase in the medium than could be accounted for by release of enzyme from the cell surface, indicating that 16% of the internalized lipoprotein lipase had been delivered to the medium.

To investigate whether degradation of lipoprotein lipase occurs in lysosomes, the effects of the lysosomotropic agent...
Lipoprotein Lipase Secretion and Degradation

Effects of chloroquine on lipoprotein lipase in adipocyte cultures

Adipocytes were incubated with heparin (10 units/ml), chloroquine (100 µM), heparitinase (0.5 units/ml), and heparinase (0.5 units/ml) for 5 h at 37 °C as indicated. Medium, cell surface, and intracellular lipoprotein lipase was measured by ELISA. Results are expressed as the mean ± S.D. from three pools of three dishes.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lipoprotein lipase</th>
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<tbody>
<tr>
<td></td>
<td>Medium</td>
</tr>
<tr>
<td>Control</td>
<td>4.81 ± 0.47</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>8.09 ± 0.12</td>
</tr>
<tr>
<td>Heparin</td>
<td>16.6 ± 0.42</td>
</tr>
<tr>
<td>Heparin + chloroquine</td>
<td>17.3 ± 1.93</td>
</tr>
<tr>
<td>Heparinase + heparitinase</td>
<td>8.15 ± 0.66</td>
</tr>
<tr>
<td>Heparinase, heparitinase + chloroquine</td>
<td>8.83 ± 1.36</td>
</tr>
</tbody>
</table>

TABLE II

Chloroquine were studied. During a 5-h incubation, chloroquine exhibited a maximal effect on intracellular and medium lipoprotein lipase at a concentration between 100 and 250 µM (Fig. 6). The intracellular lipoprotein lipase pool and the lipase accumulating in medium increased to maxima of 2- and 4-fold, respectively. A time course showed that the effects of chloroquine occur rapidly. Within 1 h of chloroquine treatment (100 µM), intracellular lipoprotein lipase increased 38% in treated cultures relative to controls. Medium lipoprotein lipase levels in chloroquine-treated dishes were 28% higher than controls by 2.5 h (data not shown). These increases in intracellular and medium lipoprotein lipase are consistent with an inhibition of enzyme degradation. The inhibition of lipase degradation by chloroquine was quantitated directly by measuring the formation of iodotyrosine. Cells were preincubated for 1 h in the presence or absence of 100 µM chloroquine. The cultures were then incubated with 125I-lipoprotein lipase at 4 °C for 30 min, loaded with 125I-lipoprotein lipase at 37 °C for 30 min, washed with heparin-containing medium at 4 °C to remove surface-associated enzyme, and finally incubated at 37 °C for up to 1 h. Chloroquine (100 µM) reduced total iodotyrosine (intracellular, cell surface, and medium) accumulation by 92% at 30 min by 83% at 1 h (Fig. 7). Chloroquine had no effect on the internalization of 125I-lipoprotein lipase. The amounts of exogenous 125I-lipoprotein lipase internalized at the end of the first 37 °C incubation were similar: 25 ng/dish for the control dishes and 30 ng/dish for cells exposed to chloroquine. Labeled lipoprotein lipase disappeared from the cell by a first-order process (Fig. 7). Removal from the intracellular compartment is due to both degradation and recycling of 125I-lipoprotein lipase to the medium. Assuming that both processes are first order, degradation rate constants can be calculated as follows:

\[ K_1 = K - K_2 \]

\[ K_2 = \frac{B_N}{A_s (1 - e^{-K t})} \]

where \( K \) is the fractional rate constant of removal of 125I-lipoprotein lipase from the intracellular compartment; \( K_1 \), the degradation rate constant; \( K_0 \), the rate constant of appearance of 125I-lipoprotein lipase in the medium; and \( A_s \) and \( B_N \) are the 125I-lipoprotein lipase radioactivity in the cells at \( t = 0 \) and in the medium at \( t = 60 \) min, respectively. The degradation rate constants were 0.78 and 0.13 h⁻¹ and the recycling rate constants were 0.10 and 0.13 h⁻¹ for control and chloroquine-treated cultures, respectively. These experiments indicate that lipoprotein lipase degradation occurs in lysosomes and that the majority of the internalized enzyme is degraded. Lipoprotein lipase degradation was minimal in both endoglycosidase-treated (Fig. 4D) and heparin-treated cultures (10). The data presented in Table II confirm these observations. There were no additive effects of chloroquine and either endoglycosidase or heparin treatment of adipocytes on medium and total lipoprotein lipase levels. In this experiment, total lipoprotein lipase levels in endoglycosidase-treated cultures were not significantly different from untreated controls. However, in a similar experiment, total lipoprotein lipase was increased by 125% in both endoglycosidase- and chloroquine-treated cultures relative to controls (data not shown). This disparity may be due in part to differences in basal lipoprotein lipase secretion rates and to differences in how quickly lipoprotein lipase synthesis rates are affected by endoglycosidase treatment.

DISCUSSION

The results of the present study are significant in that they establish a role for heparan sulfate proteoglycans in the regulation of lipoprotein lipase binding, synthesis, secretion, and degradation. Equilibrium-binding experiments show that the binding of lipoprotein lipase to the cell surface of cultured adipocytes is specific and saturable, exhibiting an association constant of 0.002 × 10⁶ M⁻¹. Pretreatment of adipocyte cultures with endoglycosidases eliminates high affinity binding sites, demonstrating that the lipase is bound to heparan sulfate proteoglycans or heparan sulfate-like molecules. Acute responses of endoglycosidase treatment include decreased binding of lipoprotein lipase to the cell surface and increased efflux of lipoprotein lipase from the cell. These changes were accompanied by a decrease in lipoprotein lipase degradation, as is evidenced by the decrease in the loss of total, 35S-labeled lipoprotein lipase in treated cultures relative to controls. Following 2.5 h of endoglycosidase treatment, the relative rate of lipoprotein lipase synthesis has also decreased.

These data support the hypothesis proposed by Cupp et al. (10) that the release of lipoprotein lipase from the cell surface of adipocytes prevents its degradation. Binding of lipoprotein lipase to the cell surface was eliminated by two distinct treatments: addition of heparin to the medium and removal of heparan sulfates with endoglycosidases. Both treatments blocked degradation and yet acted by different mechanisms. Endoglycosidases affected the integrity of lipoprotein lipase receptors, whereas heparin in the medium presumably displaced the lipase from the cell surface by competing with endogenous receptors and forming soluble heparin-lipase complexes in the medium. Experiments with chloroquine indicate that intracellular lipoprotein lipase is degraded in lysosomes. Chloroquine had no additional effect on total lipoprotein lipase levels in either heparin- or endoglycosidase-treated cultures, supporting the view that removal of lipoprotein lipase from the cell surface prevents lysosomal degrada-
Lipoprotein Lipase Secretion and Degradation

Studies with $^{125}$I-lipoprotein lipase showed that surface-bound lipoprotein lipase was internalized and degraded. A recent report from Murphy-Ullrich and Mosher (23) shows that specific receptors on the surface of endothelial cells similarly mediate thrombospondin binding and degradation. The authors present evidence suggesting that these receptors are heparan sulfate proteoglycans.

Thus, a new picture of lipoprotein lipase secretion emerges. As lipoprotein lipase is secreted, the enzyme binds to specific cell surface receptors, which are most likely heparan sulfate proteoglycans. If lipoprotein lipase is not released from the cell surface, the enzyme is internalized and either degraded in lysosomes or recycled. The net release of enzyme from the cell surface would be determined by the association constant of lipoprotein lipase for the cell surface receptor, the number of receptors, the presence of soluble lipase-binding molecules in the medium, and the residence time of the lipase-receptor complex on the cell surface. Candidates for competitors of plasma membrane heparan sulfate proteoglycans include plasma lipoproteins and soluble glycosaminoglycans. In the absence of such molecules, 76% of the synthesized lipoprotein lipase is degraded (10). The presence of molecules with high affinity for the lipase in the medium can reduce the degradation rate to 21% of the synthetic rate, as in the case of heparin (10).

Evidence for degradation of a major fraction of newly synthesized lipoprotein lipase in cultured adipocytes has also been presented for 3T3-L1 cells (24) and guinea pig adipocytes (11). Fig. 8 illustrates various pathways for the recycling of internalized lipoprotein lipase: rapid recycling of superficial endocytic vesicles; recycling from endosomes or lysosomes; and finally, transport from the Golgi after passage through endosomes or lysosomes. These various pathways have been proposed for the recycling of cell surface sialoglycoconjugates in HeLa cells (25). Which of these pathways predominates remains to be determined. Relevant to the design of a model of lipoprotein lipase transport are the observations of Conrad and co-workers (17, 26). These researchers have shown that, in a rat hepatocyte cell line, newly synthesized heparan sulfate proteoglycans are both incorporated into the pericellular matrix and secreted into the medium, and subsequently internalized from each of these compartments (17, 26). It is therefore reasonable to consider that lipoprotein lipase is bound to heparan sulfate proteoglycans during part of its intracellular transport, in organelles where pH and ionic concentrations are compatible with a tight interaction. The model presented in Fig. 8 depicts lipoprotein lipase as a constitutively secreted protein and emphasizes new potential sites for regulation of this enzyme at the level of receptor binding, internalization, degradation, and enzyme recycling. This model does not include enzyme in storage vesicles that could be released by hormonally regulated mechanisms. The experiments summarized in this report were not designed to address this issue. Olivcrona et al. (24) have presented evidence for the presence of significant amounts of inactive lipoprotein lipase in 3T3-L1 adipocytes. These researchers have suggested that this inactive pool could be a precursor of active enzyme or could result from the dissociation of active dimeric lipase to the inactive monomeric species.

In addition, the data presented in this report raise the possibility that endoglycosidases, specifically, may function in vivo to modulate the release of lipoprotein lipase from adipocytes and other cells that synthesize lipoprotein lipase. Hook and co-workers identified an endoglycosidase that was released from platelets in response to thrombin, collagen, and ADP (27). This enzyme has been purified to homogeneity and identified as a lysosomal enzyme (28). Addition of the purified enzyme to cultured bovine endothelial cells has been shown to release heparin-like activity (29). Rosenberg and co-workers (30) have proposed that this lysosomal endoglycosidase presents at low levels in the extracellular matrix of the vascular bed, functions in the maintenance of normal heparan sulfate proteoglycan pools and, subsequently, normal smooth muscle cell function and growth. Similarly, the extracellular matrix surrounding adipocytes may contain low levels of endoglycosidases that facilitate the efflux of lipase from adipocytes to the endothelium. In differentiated cultured Ob17 adipocytes, Vannier and Ailhaud (31) observed no spontaneous secretion of lipoprotein lipase. Secretion was observed only upon addition of heparin to the medium. In these studies the presence of enzyme in the medium was monitored by catalytic activity. Rapid inactivation at 37 °C of low levels of enzyme could explain the results. It is, however, possible that under the conditions of culture, no endogenous endoglycosidases or soluble heparan sulfates were present in the medium, thereby maximizing internalization and degradation.

Other effectors of cell surface and extracellular matrix heparan sulfate proteoglycans include hormones (32, 33), phosphatidylinositol-specific phospholipase C (33), and proteases (34). Conrad and co-workers (33) have reported that heparan sulfate proteoglycans in a rat hepatocyte cell line are linked to the plasma membrane via a phosphatidylinositol attachment. This attachment can be cleaved by an insulin-dependent phosphatidylinositol-phospholipase C (33). The cleaved heparan sulfate proteoglycan then can bind to a proposed inositol phosphate receptor. Preliminary studies from our laboratory show that medium and total lipoprotein lipase levels increase relative to controls in adipocyte cultures treated with myo-inositol-2-monophosphate (data not shown). Myo-Inositol-2-monophosphate would compete with "cleaved" heparan sulfate proteoglycans for binding to an inositol phosphate receptor.

The precise mechanism of the endoglycosidases' effect on lipoprotein lipase turnover and distribution remains unclear. Although endoglycosidase treatment decreased cell surface heparan sulfate proteoglycans as determined by nitrous acid degradation, biologically active degradation products may have been released into the medium as well. These degradation products would have provided soluble lipoprotein lipase.

**Fig. 8. Proposed itinerary of lipoprotein lipase.** $\bullet$ denotes newly synthesized lipoprotein lipase; $\Box$, recycled lipoprotein lipase; $t$, heparan sulfate proteoglycans; and $\square$, molecules with high affinity for lipoprotein lipase, such as heparin or plasma lipoproteins; N, nucleus; ER, endoplasmic reticulum; G, Golgi; EV, endocytic vesicle; CURL, compartment for uncoupling of receptors and ligands; and L, lysosomes.
binding sites, which could have competed with surface receptors for lipase binding. The decrease in the relative rate of lipoprotein lipase synthesis observed following 2.5 h of endoglycosidase treatment is also interesting. The absence of intact heparan sulfate proteoglycans or the presence of biologically active heparan sulfate degradation products may somehow provide a signal to decrease lipoprotein lipase synthesis. This effect of endoglycosidases is in contrast to heparin, which has no detectable effect on lipoprotein lipase synthesis.

In conclusion, we have presented evidence for the presence of a class of high affinity receptors for lipoprotein lipase on the surface of cultured adipocytes. The receptor most likely is somehow involved in providing a signal to decrease lipoprotein lipase synthesis and degradation and leads to enhanced enzyme release to the extracellular compartment.

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