Lipoprotein Lipase-mediated Selective Uptake from Low Density Lipoprotein Requires Cell Surface Proteoglycans and Is Independent of Scavenger Receptor Class B Type 1*

Lipoprotein lipase (LpL) hydrolyzes chylomicron and very low density lipoprotein triglycerides to provide fatty acids to tissues. Aside from its lipolytic activity, LpL promotes lipoprotein uptake by increasing the association of these particles with cell surfaces allowing for the internalization by receptors and proteoglycans. Recent studies also indicate that LpL stimulates selective uptake of lipids from high density lipoprotein (HDL) and very low density lipoprotein. To study whether LpL can mediate selective uptake of lipids from low density lipoprotein (LDL), LpL was incubated with LDL receptor negative fibroblasts, and the uptake of LDL protein, labeled with 125I, and cholesteryl esters traced with [3H]cholesteryl oleoyl ether, was compared. LpL mediated greater uptake of [3H]cholesteryl oleoyl ether than 125I-LDL protein, a result that indicated selective lipid uptake. Lipid enrichment of cells was confirmed by measuring cellular cholesterol mass. LpL-mediated LDL selective uptake was not affected by the LpL inhibitor tetrahydrolipstatin but was nearly abolished by heparin, monoclonal anti-LpL antibodies, or chlorate treatment of cells and was not found using proteoglycan-deficient Chinese hamster ovary cells. Selective uptake from HDL, but not LDL, was 2–3-fold greater in scavenger receptor class B type I overexpressing cells (SR-BI cells) than compared control cells. LpL, however, induced similar increases in selective uptake from LDL and HDL in either control or SR-BI cells, indicative of the SR-BI-independent pathway. This was further supported by ability of LpL to promote selective uptake from LDL in human embryonal kidney 293 cells, cells that do not express SR-BI. In Chinese hamster ovary cell lines that overexpress LpL, we also found that selective uptake from LDL was induced by both endogenous and exogenous LpL. Transgenic mice that overexpress human LpL via a muscle creatine kinase promoter had more LDL selective uptake in muscle than did wild type mice. In summary LpL stimulates selective uptake of cholesteryl esters from LDL via pathways that are distinct from SR-BI. Moreover this process also occurs in vivo in tissues where abundant LpL is present.

Lipoprotein lipase (LpL) is synthesized primarily in adipose, muscle, and heart and plays a critical role in hydrolysis of chylomicron and very low density lipoprotein triglycerides. As a result, LpL provides fatty acids to tissues as an energy source (see reviews in Refs. 1–3). LpL associates with lipoproteins and binds to cell surface proteoglycans, especially heparan sulfate proteoglycans (HSPG) (4–7), and also promotes the cellular uptake of a wide range of lipoproteins and modified lipoproteins by acting as a “bridging” molecule (8, 9). LpL-lipoprotein complexes bound to HSPG can be internalized by cell surface receptors or as a consequence of internalization of cell surface proteoglycans (10–12).

LpL-mediated bridging of cell surface proteoglycans and lipoproteins might lead to other metabolic processes. Cells obtain lipoprotein lipids via a process, selective uptake, in which cholesteryl esters from the lipoprotein core are taken up by cells without concomitant uptake of whole lipoprotein particles. LpL could also be involved in this process (13, 14). Although most studies on selective uptake have focused on HDL, LDL likely undergoes a similar process (15–17). LDL selective uptake was observed in mice, and among the sites of selective uptake of LDL lipid were tissues that express abundant LpL such as muscle, heart, and adipose (15). In contrast, selective HDL cholesteryl ester uptake occurs predominantly in atherosclerotic tissues and liver (15), tissues that are rich in scavenger receptor class B type 1 (SR-BI). There are no available data that directly show LpL-mediated LDL selective uptake.

In the current studies, we examined pathways involved in LpL-mediated selective uptake of LDL lipids. The role of SR-BI in this process was specifically examined using CHO cells that overexpress SR-BI. In addition, we tested whether endogenously synthesized LpL has similar effects on LDL selective uptake as exogenous LpL, using CHO cells that overexpress LpL. Finally the importance of LpL in this process was studied in vivo using mice that overexpress LpL in muscle. Our data show that LpL-mediated LDL selective uptake is independent of SR-BI but requires cell surface proteoglycans.

**EXPERIMENTAL PROCEDURES**

Materials—Sodium [125I]iodide (NEZ033) was purchased from NEN Life Science Products. [1α,2α-(n)-3H]cholesteryl oleoyl ether (TRK888) was purchased from Amersham Pharmacia Biotech. Bovine serum albumin and β-sitosterol were purchased from Sigma. Fetal bovine serum

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* This work was supported by National Institutes of Health Grants HL40404, HL56984, and HL45095. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**§ Supported by National Institutes of Health Training Grant HL07343-22 in Atherosclerosis Research.**

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1 The abbreviations used are: LpL, lipoprotein lipase; HSPG, heparan sulfate proteoglycans; HDL, high density lipoprotein; LDL, low density lipoprotein; SR-BI, scavenger receptor class B type I; CHO, Chinese hamster ovary; LPDS, lipoprotein-deficient serum; DMEM, Dulbecco’s modified Eagle’s medium; THL, tetrahydrolipstatin; CET, cholesteryl oleoyl ether; TC, tyramine cellbiose; GLC, gas liquid chromatography; apoB, apolipoprotein B.
was purchased from Hyclone Laboratories, Logan, UT. Lipoprotein-deficient serum (LPDS) was prepared from fetal bovine serum by sequential density gradient ultracentrifugation (d > 1.225 g/ml) followed by extensive dialysis against saline containing 1 mg/ml EDTA, pH 7.4. Isolated LPDS was filter-sterilized using a 0.45-μm filter and stored frozen at −20 °C. The LPDS was thawed and solubilized in 0.1 M sodium phosphate buffer (pH 7.0) and stored at the final concentration of 24 μmol. Two days before the experiments, cells were grown in F12 medium as Vec and SR-BI cells except there was no G418 added. CHO cells overexpressing murine SR-BI (32) were kindly provided by Dr. Martin Merkel (Rockefeller University). These mice have a human LpL minigene driven by muscle creatine kinase promoter and are denoted MCK-LpL mice. The characteristics of these mice are detailed elsewhere (2, 20, 21).

Isolation, Labeling, and Characterization of Human LDL and HDL—LDL and HDL were isolated from normolipidemic human plasma by sequential ultracentrifugation at 1.025 < d < 1.060 (LDL) and 1.080 < d < 1.210 g/ml (HDL) for 20 and 48 h (22). Isolated LDL was labeled with [3H]cholesterol oleoyl ether ([3H]CEt) by methods described by Ishikawa et al. (28). Briefly, a glass window culture vessel was filled with unlabeled LDL 0.25 mg/ml and exposed to 125I-TC dissolved in 500 μl of dichloromethane by evaporation under a nitrogen stream. One ml of LDL (8–10 mg) was mixed with 5 ml of plasma as a source of cholesterol ester transfer protein obtained from a normolipidemic human subject. The mixture was then incubated for 24 h at 37 °C to allow labeling of LDL followed by re-isolation of LDL by methods described above. Immediately after isolation, LDL was labeled with either 125I using iodine monochloride (24) or coupled with 125I-labeled tyramine cellobiose (TC). 125I-TC was prepared by using 1,3,4,6-tetrachloro-3a,6a-dihenylpyrogallol (iodogen) (0.06–0.1 mCi/mmol TC) (25, 26) and covalently linked to human LDL (5–10 nmol of TC/mg of LDL protein) after activating the iodinated TC with cyanuric chloride (25, 26) and covalantly linked to human LDL (5–10 nmol of TC/mg of LDL protein) after activating the iodinated TC with cyanuric chloride (25, 26). These miceKey Features

- LpL increases selective uptake of LDL.
- LpL increases cholesterol delivery by 3-4 fold.
- LDL cholesterol was measured in cell culture.
- Statistical significance was determined using two-tailed paired student's t tests.

Results

LpL Stimulates LDL Selective Uptake and Cholesterol Accumulation—To test whether LpL stimulates selective uptake in LDL receptor negative fibroblasts, double-labeled LDL was incubated in the presence or absence of LpL, 4 and 8 h at 37 °C. Table 1 summarizes the effect of LpL on selective uptake from LDL at two different time points. After 4 h LpL increased cholesteryl ester and apoB uptake >2-fold. Greater than 90% of both labels was not heparin releasable (100 IU/ml heparin). The upper organic solvent phase contained [3H]CEt with less than 5% 125I contamination. The solvent phase was then dried under a nitrogen stream and counted for [3H] with a Wallac model 4909 scintillation counter. Two ml of the bottom layer was transferred to a tube for 125I counts. Large organs such as liver were solubilized as described and diluted 10-fold before proceeding to phase separation.

Statistical Analysis—Two-tailed paired student's t tests were performed to determine statistical significance of all in vitro studies. In vivo studies were analyzed using two-tailed student's t tests within the group, whereas the noncomparative student's t test assuming equal variance was utilized to determine the statistical significance between groups.

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**Table I**

_Uptake of cholesterol from double-labeled LDL in LDL receptor negative human fibroblasts_

| 4-h Incubation | 8-h Incubation |
|----------------|----------------|
| LDL | LpL + LDL | LDL | LpL + LDL |
| [3H]CEt | 125I-apoB | Mass | [3H]CEt-125I-apoB | [3H]CEt | 125I-apoB | Mass | [3H]CEt-125I-apoB |
| μg C/mg P | μg/mg | μg | μg/mg |
| LDL | 14.08 ± 3.80 | 6.22 ± 0.21 | 0.40 ± 0.80 | 7.86 |
| LDL + LpL | 42.32 ± 8.60 | 11.89 ± 1.90 | 38.75 ± 5.20 | 30.43 |
| LDL | 24.54 ± 0.72 | 8.64 ± 0.87 | ND | 15.9 |
| LDL + LpL | 59.24 ± 1.49 | 14.98 ± 0.44 | ND | 45.16 |

Cellular-free cholesterol remained unchanged. Thus, the mass data confirm true cholesterol net transfer and not just exchange of radiolabel.

Free fatty acids stimulate LDL uptake in cultured cells (37). Although LDL has few triglycerides, it is possible that liberated fatty acids and/or lysophospholipids from hydrolysis of LDL triglyceride or phospholipid (38) by LpL promote selective uptake. To assess this possibility, cells were incubated with LDL and LpL in the presence or absence of THL. THL is a potent inhibitor of LpL catalytic activity, and the concentration used in the study (100 μM) almost totally inhibits LpL-mediated lipolysis of triglyceride-rich particles (39). In the presence of LpL, THL did not affect either [3H]-CEt or 125I-apoB uptake, and therefore, did not change selective uptake (Fig. 1). Thus, the effect of LpL on selective uptake is not dependent upon its catalytic activity.

Cell Surface Proteoglycans Are Involved in LpL-mediated LDL Selective Uptake—We next determined if impairing LpL binding to lipoproteins inhibits LpL-mediated LDL selective uptake. Cells were incubated with LDL and LpL in the presence of monoclonal antihuman LpL antibodies that inhibit the association of LpL with LDL particles (19). In the absence of antibodies, ~40 μg/mg cell protein cholesterol ester were delivered via selective uptake (Fig. 2). Antihuman LpL antibodies reduced selective uptake by ~80%. Incubation of irrelevant antihuman IgG did not inhibit selective uptake (data not shown), indicating binding of LpL to LDL particles is required for subsequent selective uptake. The role of cell surface proteoglycans was examined by several methods. Heparin inhibits binding of LDL-LpL complexes to cell surface proteoglycans. Incubation of heparin with LDL and LpL inhibited LpL selective uptake by fibroblasts >90%. Treating cells with sodium chloride removes glycosaminoglycan chains from cell surface proteoglycans. Chlorate incubation nearly abolished the LpL-mediated LDL selective uptake (Fig. 2).

To determine the role of cell surface proteoglycans, we performed experiments in the presence or absence of Ca2+. Depletion of Ca2+ does not affect particle binding to cell surface proteoglycans (12). The absence of Ca2+ had no effect on LpL-stimulated selective uptake from LDL (Fig. 2, inset). The role of cell surface proteoglycans was further assessed with CHO cells void of cell surface proteoglycans (PG-). Basal LDL uptake was very similar to control cells, but incubation with LpL stimulated significantly less total LDL uptake. Furthermore, there was no LpL-mediated selective uptake in this cell line (Fig. 3). Thus, selective uptake from LDL requires LpL binding to LDL and binding of LDL-LpL complexes to cell surfaces via interaction with proteoglycans.

The Contribution of SR-BI to LpL-mediated Selective Uptake in CHO Cells—LpL-mediated selective uptake is independent of Ca2+2, excluding the potential role of classical receptors. However, the process may be mediated by CLA-1 (40), the human homologue of SR-BI; model phospholipid liposomes do bind to SR-BI in the absence of Ca2+ (41). LpL-mediated LDL selective uptake was studied in CHO cells overexpressing SR-BI (SR-BI cells). SR-BI and vector-transfected control cells (Vec) were incubated with double-labeled LDL in the presence or absence of LpL at 37 °C. The uptake of LDL [3H]CEt and 125I-apoB in SR-BI and Vec cells is summarized in Table II. SR-BI overexpression increased 125I-apoB cell association as described by others (42). Thus, in the absence of LpL total LDL cholesteryl ester uptake was ~2-fold higher in SR-BI cells compared with Vec cells; however, there was little, if any, selective uptake in either cell. The addition of LpL to the medium increased the uptake of 125I-apoB and especially [3H]CEt in Vec and SR-BI cells. Both cell lines showed similar increases in LpL-mediated selective uptake with LDL, indicating LpL-stimulated selective uptake. As was found with fibroblasts, treatment with chlorate reduced total LDL uptake to control levels, and LpL-associated selective uptake was nearly abolished. Of note, the contribution of selective uptake to total LDL cholesteryl uptake was relatively small in these cells compared with LDL receptor negative fibroblasts, presumably because most LDL was taken up by the LDL receptors on the surface of SR-BI and Vec cells. Nonetheless, these experiments demonstrate that LpL-mediated selective uptake is not enhanced by overexpression of SR-BI.

To determine the ability of SR-BI to modulate cell cholesterol mass by whole particle uptake versus selective uptake, selective uptake was also determined using actual and predicted net cholesterol mass accumulation based on GLC mass measurements and 125I-apoB uptake, respectively (Fig. 4). It was predicted that if there was selective cholesteryl ester uptake from LDL, measured net cholesterol mass accumulation would exceed predicted values generated from 125I-apoB uptake and degradation. As a control for active SR-BI, 125I-labeled HDL, known to undergo increased selective uptake via SR-BI (43), was incubated under identical experimental settings. Fig. 4 compares cholesterol accumulation via selective uptake from LDL and HDL. Cells that were incubated with LDL in the absence of LpL showed little cholesterol mass accumulation, suggesting LDL alone was not sufficient to promote substantial selective uptake, consistent with results seen in LDL receptor negative fibroblasts. In contrast, the addition of LpL to the medium increased cholesterol accumulation via selective uptake in both Vec and SR-BI cells. In parallel experiments with HDL, there was no cholesterol accumulation from selective uptake in all cell lines. SR-BI cells showed 2–3-fold higher cholesterol mass accumulation compared with Vec cells. The addition of LpL further increased selective cholesterol mass accumulation from HDL by 1.5–2-fold, with both Vec and SR-BI showing similar relative increases. Taken together, the cells studied herein, SR-BI facilitates selective uptake from HDL but not LDL.

Although our results indicated that LpL induced selective uptake from LDL via a SR-BI-independent pathway, it was still possible that small amounts of SR-BI expressed by CHO cells
contributed. To explore this possibility, human embryonal kidney 293 cells that totally lack SR-BI (30) were used under similar experimental conditions. As shown in Fig. 5, incubation of the cells with LDL alone showed no selective uptake. However, when LDL was incubated in the presence of 100 nM LpL, selective uptake from LDL was substantial. Selective uptake from LDL in the presence of LpL was abolished when cells were pretreated with chlorate, providing further support for an important role of cell surface proteoglycans in mediating selective uptake in the absence of SR-BI.

Selective Uptake from LDL Is Promoted by Both Endogenous and Exogenous LpL—LpL stimulates selective uptake from LDL via a pathway that involves cell surface proteoglycans. We questioned whether endogenously expressed LpL would have similar effects on selective uptake from LDL. To address this, experiments were performed using CHO cells that endogenously overexpress LpL. Results are summarized in Table III. When wild type CHO cells were incubated with LDL without exogenous LpL for 4 h, they showed similar cholesteryl ester uptake (calculated from either \(^{125}\text{I}\)-apoB or \([^{3}\text{H}]\text{CEt}\) uptake) with values similar to that of the CHO control cells in Table II. On the other hand, in the experiments using LpL overexpressing cells, LDL selective uptake markedly increased despite the lack of exogenous LpL. This process was proteoglycan-dependent as chlorate abolished selective uptake from LDL in endogenously LpL expressing cells. These studies indicate that endogenous LpL also stimulates selective uptake from LDL, a pathway of...
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FIG. 3. Selective cholesteryl ester uptake from LDL in proteoglycan-deficient and control CHO cells. Cells were incubated with 100 μg/ml of double-labeled LDL with or without 100 nM LpL for 4 h to assess cholesteryl ester delivery based on [3H] and 125I counts. At the end of experiments, cholesteryl ester uptake from LDL was estimated based on 125I-apoB and [3H]CEt counts using the ratios of cholesteryl ester to protein for each LDL used. Selective uptake of cholesteryl esters is indicated in control (Vec) and cells that do not express proteoglycans (PG-) and was determined by subtracting 125I-apoB values from [3H]CEt. Results are shown as the mean of triplicate determinations ± S.D. of a representative experiment.

FIG. 4. Cholesterol mass delivery via selective uptake from LDL and HDL in control (Vec) and SR-BI overexpressing cells (SR-BI) for LpL in stimulating LDL selective uptake in vivo.

Effect of LpL and chlorate on uptake of cholesteryl ester from LDL in control cells (Vec) and SR-BI overexpresser cells (SR-BI)

Cells were grown in the medium containing 5% fetal bovine serum. Two days before the experiment, medium was replaced with fresh medium containing 10% LPDS in the presence or absence of 50 mM sodium chlorate (ClO3) and incubated for an additional 36 h at 37°C to inhibit sulfation of proteoglycans. On the day of the experiment, 100 μg/ml double-labeled LDL was incubated with (+ LpL) or without 100 nM LpL for 4 h to assess cholesteryl ester delivery based on [3H] and 125I counts. For chlorate-treated cells, chlorate was included during experiments to inhibit sulfation of newly synthesized proteoglycans. At the end of experiments, cholesteryl ester uptake from LDL was estimated based on 125I-apoB and [3H]CEt counts using the ratios of cholesteryl ester to protein for each LDL used. SU indicates selective uptake of cholesteryl esters in control (Vec) and SR-BI overexpressing (SR-BI) cells and was determined by subtracting 125I-apoB values from [3H]CEt. Results are shown as the mean of triplicate wells ± S.D. * indicates statistically significant differences between uptakes with or without LpL at p < 0.01.

TABLE II

| Treatment          | 125I-apoB SU | [3H]CEt SU | 125I-apoB SU | [3H]CEt SU |
|--------------------|--------------|------------|--------------|------------|
| LDL                | 33.97 ± 3.06 | 37.55 ± 1.34 | 3.58 ± 1.78  | 54.04 ± 3.51 | 57.63 ± 2.96 | 3.59 ± 2.20 |
| LDL + ClO3         | 19.91 ± 0.55 | 24.11 ± 3.75 | 4.19 ± 3.58  | 36.84 ± 3.86 | 37.39 ± 4.53 | 0.55 ± 3.51 |
| LDL + LpL          | 66.47 ± 5.52 | 83.63 ± 2.74a | 17.15 ± 6.75a | 112.11 ± 6.61a | 131.48 ± 4.82a | 19.37 ± 4.63 |
| LDL + LpL + ClO3   | 27.73 ± 2.97 | 29.99 ± 3.39 | 2.25 ± 5.18  | 37.20 ± 2.95 | 38.90 ± 5.88 | -1.16 ± 3.16 |

potential physiological relevance in tissues and cells where abundant LpL is present.

LpL Increases LDL Selective Uptake in Tissues That Overexpress Human LpL—To study LpL-stimulated LDL cholesteryl ester selective uptake in vivo, [3H]CEt and 125I-tyramine cellobiose apoB (125I-TC-apoB) -labeled LDL were injected into MCK-LpL and littermate control mice. The delivery of labeled LDL from plasma to different tissues over 24 h in these mice is shown in Table IV. Almost all organs, except kidney, showed some degree of selective uptake regardless of genotype. Adrenal glands had 2–5-fold more cholesteryl ester uptake than apoB, consistent with a role for LpL in stimulating LDL selective uptake in vivo.

DISCUSSION

In the current studies, we showed that LpL augmented selective uptake of LDL cholesterol in LDL receptor negative human fibroblasts and CHO cells. In comparison with other selective uptake studies, a relatively high but physiologic LDL concentration (100 μg/ml) was used to ensure saturation of LDL receptors in CHO cells and significant proteoglycan-mediated uptake of LDL in receptor-negative fibroblasts (45). When LpL was present, uptake of the protein moiety (125I-apoB) increased 2–3-fold, and cholesteryl ester delivery was 2–5-fold higher than could be accounted for by the total LDL particle uptake. Increased LpL-mediated net cholesterol delivery via selective uptake was also shown by actual cholesterol mass determination.

The pathways responsible for LpL-mediated selective uptake...
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Fig. 5. Effect of LpL and chlorate on selective cholesteryl ester uptake from LDL in human embryonal kidney 293 cells that do not express SR-BI. Cells were incubated with 100 μg/ml of double-labeled LDL for 4 h to assess cholesteryl ester delivery based on [3H] and 125I counts. In similar experiments, cells were pretreated with sodium chlorate as described under "Experimental Procedures" and Fig. 2. At the end of experiments, cholesteryl ester uptake from LDL was estimated based on 125I-apoB and [3H]CEt counts using the ratios of cholesteryl ester to protein, and selective uptake of cholesteryl esters was determined by subtracting 125I-apoB values from [3H]CEt. Results are shown as the mean of triplicate determinations ± S.D. of a representative experiment.

Table III

Uptake of cholesteryl esters from LDL in control CHO cells and LpL overexpressor cells (LpLOx)

Cells were incubated with or without 50 mM sodium chlorate (ClO3) as described under "Experimental Procedures." On the day of the experiment, 100 μg/ml double-labeled LDL was incubated for 4 h to assess cholesteryl ester (CE) delivery. At the end of experiments, cholesteryl ester uptake from LDL was estimated based on 125I-apoB and [3H]CEt counts using the ratios of cholesteryl ester to protein. Results are shown as the mean of triplicate wells ± S.D. * indicates statistically significant differences between CE uptakes calculated from 125I and [3H]CEt (p < 0.01). SU, selective uptake.

| Treatment | CHO uptake determined by | LpLOx CE uptake determined by |
|-----------|--------------------------|-------------------------------|
|           | 125I-apoB | [3H]CEt | SU | 125I-apoB | [3H]CEt | SU |
| LDL       | 36.32 ± 0.38 | 38.55 ± 5.78 | 2.22 ± 2.82 | 48.56 ± 0.38 | 58.08 ± 1.61 | 9.52 ± 2.67* |
| LDL + ClO3 | 31.03 ± 0.09 | 29.84 ± 1.38 | -1.19 ± 2.18 | 19.42 ± 0.11 | 17.74 ± 1.70 | -1.68 ± 0.73 |

Table IV

Percent organ distribution of injected LDL in control and MCK-LpL mice

Uptake of [3H]cholesteryl oleoyl ether and 125I-tyramine cellulose tracers from human LDL was determined in control and MCK-LpL mice as described under "Experimental Procedures." Total counts of LDL injected (200 μg of protein) were divided by accumulated counts in each organ to show the percent of plasma LDL uptake/organ after 24 h. Total muscle uptake was calculated based on the assumption that muscle accounts for 42% of body weight. The results are expressed as percent of LDL uptake/organ ± S.E. * indicates a significant difference between control and MCK-LpL mice, p < 0.001.

| Treatment | 125I | [3H] | MCK-LpL (n = 9) |
|-----------|------|------|----------------|
| Kidney    | 2.62 ± 1.08 | 1.26 ± 0.39 | 2.18 ± 0.53 | 1.24 ± 0.17 |
| Heart     | 0.27 ± 0.05 | 0.56 ± 0.07 | 0.36 ± 0.05 | 0.40 ± 0.10 |
| Lung      | 0.64 ± 0.26 | 1.15 ± 0.18 | 0.69 ± 0.25 | 1.15 ± 0.19 |
| Liver     | 38.18 ± 4.99 | 66.97 ± 7.62 | 34.52 ± 5.12 | 67.79 ± 7.04 |
| Adrenal   | 0.13 ± 0.05 | 0.69 ± 0.36 | 0.12 ± 0.04 | 0.64 ± 0.17 |
| Muscle    | 11.67 ± 6.68 | 12.77 ± 7.48 | 14.26 ± 3.49 | 26.36 ± 5.48* |

were defined. LpL-enhanced LDL selective uptake did not require LpL catalytic activity, because THL did not abolish selective uptake from LDL particles. On the other hand, impairing the ability of LpL to bind either LDL particles or cell surface proteoglycans, either by heparin or anti-LpL antibodies, significantly reduced selective uptake, suggesting that the bridging property of LpL was essential for selective uptake to occur. LpL-induced selective uptake can be stimulated by not only exogenous LpL but also by endogenously cell-synthesized LpL. Selective uptake of LDL cholesterol by fibroblasts occurred in the absence of calcium indicating that the process did not involve classical receptors. Elimination of sulfated glycosaminoglycans with chlorate or use of glycosaminoglycan negative cells nearly abolished LpL-mediated LDL selective uptake. Therefore selective uptake required cell surface proteoglycans.

Although we did not test which proteoglycans are required for LDL selective uptake, studies by a number of laboratories have shown that cell surface HSPG is the primary site for binding of LpL-LDL complexes (9, 12, 46, 47). Thus, we propose that binding of the LpL-LDL complex is mediated by cell surface HSPG. Furthermore, this anchoring process may facilitate the cholesteryl ester transfer from the LDL core to the plasma membrane, a putative acceptor for cholesteryl esters, by reducing the barrier of hydrophobic lipid movement in an aqueous environment.

The importance of cell surface proteoglycans in selective uptake was also demonstrated in hepatic lipase-induced HDL selective uptake. Ji et al. (48) showed that the addition of exogenous hepatic lipase stimulated HDL selective uptake via a heparinase-sensitive pathway that was not affected by 39-kDa receptor-associated protein, an inhibitor of ligand binding to LDL receptor-related protein. ApoE, another well described ligand for HSPG, has been shown to promote LDL selective uptake. Swarnakar et al. (17) have shown that endogenously expressed apo E in mouse adrenocortical cells promotes selective uptake from LDL but not HDL. The studies suggested that selective uptake pathways of LDL and HDL in apoE expressing adrenocortical cells are different. LDL selective uptake was mediated by a low affinity, high capacity cell surface proteoglycan pathway, whereas HDL selective uptake was mediated by SR-BI. Cholesterol delivery from LDL via selective uptake was quantitatively greater than that of HDL, suggesting selective uptake from LDL also plays an important role in cholesterol delivery into these cells.

The potential role of CLA-1 on LpL-mediated selective uptake was tested by using CHO cells that overexpress SR-BI. SR-BI and CLA-1 share similar characteristics (40), and thus similar results would be expected with CLA-1. SR-BI overexpressing cells had no greater LpL-mediated LDL selective uptake than did control cells; this suggests that selective uptake is SR-BI independent, at least in CHO cells. Although total
cholesteryl ester uptake was greater in CHO cells than LDL receptor negative fibroblasts, cholesteryl ester delivery via selective uptake was similar between CHO cells, which have LDL receptors, and LDL receptor negative fibroblasts (20–30 μg of cholesteryl ester/mg of cell protein). In the presence of LpL, most cholesteryl ester accumulating in LDL receptor fibroblasts came primarily from LDL selective uptake; the contribution of selective uptake over total cholesterol uptake was less in CHO Vec and SR-BI CHO cells (compare Tables I and II). This suggests that LDL receptors are the major pathway for LDL whole particle uptake and subsequent cholesterol accumulation. Based on our studies, however, we hypothesize that selective uptake can be an alternative pathway for cholesteryl ester accumulation when LDL receptors are sparse or down-regulated, such as in peripheral tissues (49).

Swarnakar et al. (42) showed that selective uptake from LDL occurred via cell surface proteoglycans in Cos cells, cells that have little or no SR-BI. This indicates that cell surface proteoglycans might mediate selective uptake from LDL even in the absence of LpL. Our studies are consistent with their observation, and we showed that the presence of LpL further promoted cholesteryl ester delivery by this pathway. Still, our studies in SR-BI overexpressing CHO cells demonstrated little or no SR-BI-mediated selective uptake from LDL, different than results of Swarnakar et al. (42) where SR-BI-transfected Cos cells had significant selective uptake from LDL via SR-BI. One possibility for these disparate conclusions is differences in metabolism of LDL particles between CHO and Cos cells. CHO cells used herein efficiently internalized and degraded LDL particles by efficient endocytic pathways; the majority of cholesteryl esters is internalized by this pathway in our transfected cells. Thus, although small levels of selective uptake may occur via SR-BI in these cells, cholesteryl ester delivery via selective uptake is likely masked by the large amounts of cholesteryl esters entering via endocytic pathways. In contrast, the Cos cells used by Swarnakar et al. (42) had —250-fold less internalization of LDL particles (~0.2 μg of LDL cholesteryl esters/mg of cell protein in Cos cells compared with 52 μg/mg cell protein in CHO cells at LDL concentrations of 100 μg/mL). Under these conditions, overexpression of SR-BI could contribute substantially to LDL cholesteryl ester delivery through selective uptake as an alternate route for cholesterol delivery. Similarly, SR-BI appears to stimulate selective uptake from LDL particles in steroidogenic cells. Because these cells utilize large amounts of cholesterol/cholesteryl esters, the presence of SR-BI may potentiate cholesteryl ester delivery from not only HDL, but also from LDL particles to meet needs for steriodogenesis. Therefore, differences in cell cholesterol/cholesteryl ester needs, metabolism, and trafficking may contribute to different cellular mechanisms that can contribute to varying results on the role of SR-BI in different cell lines.

We also studied selective uptake in LDL receptor negative fibroblasts that do not require high amounts of cholesteryl esters under normal physiological conditions. Selective uptake via SR-BI had little or no functional role in this cell line. In the presence of LpL, however, these cell lines exhibit selective uptake from both LDL and HDL via pathways involving cell surface proteoglycans. It is possible that this mechanism provides an additional pathway for cholesteryl ester delivery to cells such as LpL-expressing cells, e.g. macrophages, and may contribute to pathological accumulation of cholesteryl esters, a possible atherogenic pathway.

HDL selective cholesterol uptake was primarily mediated by SR-BI (50). However, when LpL was added to medium, HDL-selective uptake was increased in VEC and SR-BI, but not in PG-cells. These data confirm that of others (18, 19) showing that LpL-enhanced HDL selective uptake also requires cell surface proteoglycans (13). Thus, although the mechanisms of LDL and HDL selective uptake differ in the absence of LpL, enhanced selective uptake in the presence of LpL requires cell surface proteoglycans for both LDL and HDL.

Glass et al. (44) and Green and Pittman (15) showed that tissue sites of selective uptake in rats are different for HDL and LDL. Selective uptake occurred mainly in steroidogenic tissues and liver, whereas LDL selective uptake was also seen in LpL-rich tissues such as muscle, heart, and adipose tissues. This suggested that the pathways of selective uptake for these particles are different. Using mice overexpressing human LpL specifically in muscle, we found that adrenal and liver accumulated more [3H]CEt than 125I, indicating cholesteryl esters from LDL were selectively transferred to these organs. However, overexpression of LpL in muscle led significantly more muscle selective uptake. Recent studies by Merkel et al. (51) also showed that muscle-specific overexpression of inactive LpL in mice resulted in higher uptake of very low density lipoprotein cholesteryl ester than could be accounted for the total metabolism of very low density lipoprotein particles.

Thus, our studies indicate that LpL enhances LDL cholesterol uptake via selective uptake pathways. Selective removal of core cholesteryl esters from LDL could lead to formation of small, dense LDL particles, particles reported to have more atherogenic properties than normal LDL (45, 52, 53). In addition, LpL-mediated LDL selective uptake could be another pathway for cholesterol delivery to LpL-rich areas such as atherosclerotic regions of the arterial wall.

Acknowledgments—We gratefully acknowledge Dr. Marin Merkel for providing MCK-LpL transgenic mice and Drs. Alan Tall, Yong Ji, and Franz Rinninger for SR-BI overexpressing cells and their critical discussions during the preparation of this manuscript. Also we thank Inge Hansen and Fannie Keyserman for their excellent technical assistance.

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