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High-Affinity Uptake and Degradation of Apolipoprotein E Free High-Density Lipoprotein and Low-Density Lipoprotein in Cultured Porcine Hepatocytes†

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ABSTRACT: Isolated pig liver membranes contain a specific "lipoprotein binding site" that recognizes low-density lipoprotein (LDL) and apolipoprotein E (apoE) free high-density lipoprotein (HDL) [Bachorik, P. S., Kwiterovich, P. O., & Cooke, J. (1978) *Biochemistry 17*, 5287–5299]. We report here that a similar site exists in cultured porcine hepatocytes and that it mediates the uptake and degradation of apoE-free HDL. The binding of ^{125}I-labeled HDL and ^{125}I-labeled LDL (^{125}I-HDL and ^{125}I-LDL, respectively) at 4 °C and the uptake and degradation of the lipoproteins at 37 °C were time dependent and saturable and were not inhibited by unrelated proteins. Chloroquine (6×10^{-5} M) inhibited the degradation of ^{125}I-HDL by 76% and of ^{125}I-LDL by >99%; leupeptin inhibited the degradation of both lipoproteins by about 25%. ^{125}I-HDL binding (4 °C), uptake, and degradation (37 °C) were inhibited by LDL, methyl-LDL, and methyl-HDL about as well as by unlabeled HDL but were unaltered in Pronase-treated cells or in cells that were cultured for 24 h in either lipoprotein-free medium or medium containing HDL or LDL (200 μg/mL). In contrast, these conditions affected the uptake and degradation of ^{125}I-LDL disproportionately. HDL and methyl-LDL inhibited ^{125}I-LDL uptake by 50% or more but had little effect on degradation. ^{125}I-LDL binding was reduced by 12% and degradation by 57% in Pronase-treated cells. Preincubation of the cells with LDL (200 μg/mL) reduced uptake by 35% and degradation by 68%. Similar preincubation with HDL (200 μg/mL) increased ^{125}I-LDL degradation by 60% but did not affect ^{125}I-LDL uptake. The findings indicated the presence in porcine hepatocytes of at least two distinct sites for lipoproteins. One site resembled the LDL receptor and mediated ^{125}I-LDL degradation. A second, Pronase-insensitive site recognized both HDL and LDL. This site mediated almost all of the degradation of ^{125}I-HDL but little if any degradation of ^{125}I-LDL.

Plasma low density (β) lipoprotein (LDL)[1] is an end product of the catabolism of VLDL (Havel, 1980) and constitutes an important source of cholesterol for many tissues. The metabolism of LDL has been studied in various types of cultured cells. Studies in human fibroblasts elucidated a receptor-mediated pathway in which LDL is bound to a specific cell surface receptor, internalized, transported to lysosomes, and degraded (Goldstein & Brown, 1977; Brown et al., 1979). During this process, cholesterol released from the lipoprotein

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[1] Abbreviations: VLDL, very low density lipoprotein(s); LDL, low-density lipoprotein(s); HDL, high-density lipoprotein(s); ^{125}I-LDL and ^{125}I-HDL, iodine-125-labeled LDL and HDL; LPDS, lipoprotein-deficient serum; FCS, fetal calf serum; EDTA, disodium ethylenediaminetetraacetate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; Cl_3CCOOH, trichloroacetic acid; PBS, phosphate-buffered saline; apoE, apolipoprotein E; $NaDodSO_4$, sodium dodecyl sulfate.

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is available for membrane synthesis. In the presence of LDL, receptor synthesis is suppressed, and the entry of LDL into the cell is limited. When starved of cholesterol, however, the cells compensate in part with increased receptor synthesis and increased receptor-mediated LDL catabolism. Less is known about the metabolism of HDL. HDL participates in the esterification of cholesterol during the initial stages of the catabolism of triglyceride-rich lipoproteins (Brown et al., 1981) and may also function in part to transport cholesterol to the liver. In some cultured cells, HDL has been found to stimulate the removal of cholesterol Stein et al., 1976; Ho et al., 1980; Stein et al., 1975; Glomset, 1968; O'Malley et al., 1981).

The liver is important in the catabolism of LDL (Slater et al., 1980; Portman et al., 1979; Calvert et al., 1975; Pittman et al., 1979a,b, 1980; Attie et al., 1980), although the mechanisms by which LDL may interact with the liver are not entirely clear. The liver accumulates injected LDL (Slater et al., 1980; Sniderman et al., 1975; Portman et al., 1979; Calvert et al., 1975), and studies in rabbits (Slater et al., 1980) and estradiol-treated rats (Chao et al., 1979) suggest that approximately half the uptake of LDL is mediated by a mechanism similar to the LDL receptor. High-affinity uptake and degradation of LDL have been observed in suspended rat (Van Berkel et al., 1980; Ose et al., 1980a) and rabbit hepatocytes (Soltys & Portman, 1979). Pangburn et al. (1981) recently presented evidence in cultured swine hepatocytes for the degradation of ^{125}I-LDL by an LDL receptor mediated mechanism. The mechanisms by which the apoproteins of HDL are catabolized and the role of the liver in this process are unclear. Several studies (Siggurdsson et al., 1979; Van Tol et al., 1978) suggested that most of the catabolism of HDL apoproteins occurs extrahepatically. Other studies indicate that the liver may contribute significantly to the catabolism of HDL. The liver accumulates injected HDL (Ose et al., 1979; Eisenberg et al., 1973; Roheim et al., 1971; Nakai & Whayne, 1976), and HDL associates primarily with parenchymal cells (Rachmilewitz et al., 1972). Suspended rat hepatocytes and nonparenchymal cells apparently bind HDL to high-affinity sites in the cell membrane, and binding is associated with the lysosomal degradation of HDL–protein (Ose et al., 1979, 1980a, 1981; Nakai et al., 1976; Van Berkel et al., 1980).

Studies in liver microsomes suggest the existence of more than one kind of high-affinity site for lipoproteins. Rat liver microsomes bind little LDL, but LDL receptor like binding activity is stimulated in rats treated with 17-α-ethinylestradiol (Kovanen et al., 1979; Windler et al., 1980). Liver microsomes from young dogs bound LDL primarily to LDL receptor like sites (Kovanen et al., 1981; Hui et al., 1981). These sites were absent in adult dogs but were induced by cholestyramine treatment (Hui et al., 1981). Liver microsomes from immature and adult dogs contain a second site that recognized apoE-containing lipoproteins but not LDL (Hui et al., 1981). Much of the LDL binding in rabbit liver microsomes was to an LDL receptor like site, but LDL also bound to a second site with different properties (Kita et al., 1981).

We found that most of the high-affinity LDL binding in pig liver plasma membranes was to sites whose properties differed from those of the LDL receptor. The peripheral LDL receptor recognizes apolipoprotein B (apoB) and apoE (Goldstein & Brown, 1977; Mahley et al., 1977a; Mahley & Innerarity, 1978; Innerarity & Mahley, 1978; Pitas et al., 1979; Innerarity et al., 1980) but not apoA-I or apoA-II (Mahley & Innerarity, 1977). Binding requires Ca^{2+} and intact lysine and arginine residues in the apoproteins, and the receptor is inactivated by proteolytic enzymes (Mahley et al., 1977b; Weisgraber et al., 1978; Goldstein & Brown, 1974). In pig liver membranes, the high-affinity ^{125}I-LDL binding site recognized apoE-free HDL, was not inactivated by Pronase (Bachorik et al., 1976, 1978), and did not require intact arginine or lysine residues in LDL (Bachorik et al., 1981). In view of its unusual lipoprotein specificity, we called the site a "lipoprotein binding site".

We examined here the relation between the uptake and degradation of LDL and HDL in cultured porcine hepatocytes. Evidence was found for at least two kinds of high-affinity sites. One site was similar to the peripheral LDL receptor and mediated the lysosomal degradation of LDL. The other site resembled the lipoprotein binding site in that it recognized LDL and apoE-free HDL. This site mediated little if any LDL degradation but appeared to account for all of the high-affinity HDL degradation that occurred in the cells. A preliminary account of some of these findings has appeared (Franklin et al., 1981).

Materials and Methods

Materials

Leupeptin, chloroquine fetuin, and neuraminidase (*Clostridium perfringens*) bound to agarose were obtained from Sigma Chemical Co., St. Louis, MO. [^{3}H]Leucine and carrier-free ^{125}I$^-$ were purchased from Amersham-Searle, Arlington Heights, IL), and [U-^{14}C]sucrose (673 mCi/mmol) was obtained from New England Nuclear, Boston, MA. Pronase was obtained from Calbiochem, San Diego, CA, and collagenase (type II) was purchased from Worthington Biochemical Corp., Freehold, NJ. Eagle's minimum essential medium and PBS were purchased from Grand Island Biological Co., Grand Island, NY. Amberlite MB-3 was obtained from Mallinkrodt, Inc., St. Louis, MO.

Methods

Preparation of Porcine Lipoproteins. LDL (d = 1.019–1.063 g/mL), HDL (d = 1.12–1.16 g/mL), and LPDS (d > 1.25 g/mL) from the blood of normolipidemic adult Duroc or Yorkshire swine were isolated (Havel et al., 1955) and dialyzed against three changes (4 L each) of 0.15 M NaCl containing 0.05% EDTA, pH 7.4. LDL and HDL were not contaminated by serum proteins or other lipoproteins as judged by methods decribed previously (Bachorik et al., 1978). The lipoproteins were prepared freshly for each experiment. LDL, HDL, and LPDS were sterilized by passage through 0.45-μm filters and stored at 4 °C (LDL and HDL) or −70 °C (LPDS) until needed. Protein concentrations were determined by the method of Lowry et al. (1951), and cholesterol concentrations were determined by the methods of the Lipid Research Clinics Program (Manual of Laboratory Operations, 1974).

Affinity Chromatography. HDL was subjected to heparin–Sepharose affinity chromatography (Weisgraber & Mahley, 1980) to remove possible traces of apoB- and apoE-containing lipoproteins. The nonadsorbed fraction was recovered and dialyzed against 0.15 M NaCl containing 0.05% EDTA.

Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis was performed as described by Weber & Osborn (1969). ApoA-I was the major apoprotein in HDL. ApoA-II appears to be absent from porcine HDL (Knipping et al., 1975; Mahley et al., 1975) and was also not observed here. Traces of unidentified proteins with apparent molecular weights of 66 500 and 58 000 were also found. ApoE was not detected in HDL, but heparin–Sepharose treatment removed traces of a high molecular weight component, possibly apoB,

that was apparent at high protein load (50–100 μg of protein/gel) and a rapidly migrating component, possibly apoC. ApoB was the major apoprotein component of LDL. Trace amounts of several unidentified components in LDL were observed, including on occasion one with an electrophoretic mobility similar to that of apoE. ApoA-I was not observed in LDL under conditions in which apoA-I would be detected in amounts less than 1% of the protein applied to the gel.

Radioiodination of LDL and HDL. LDL and heparin–Sepharose-treated HDL were labeled with ^{125}I (McFarlane, 1958; Bilheimer et al., 1972). Over 99% of the radioactivity in both lipoproteins was precipitated with Cl_3CCOOH; 1–3% of the radioactivity of ^{125}I-LDL and 3–6% of the radioactivity of ^{125}I-HDL were associated with lipids. $NaDodSO_4$–polyacrylamide gel electrophoresis revealed that 84–88% of the radioactivity in ^{125}I-HDL was associated with apoA-I, and 4–10% was associated with the minor unidentified protein components described above. No radioactivity was present in the area of the gels where apoB or apoE migrates. Approximately 95% of the radioactivity in ^{125}I-LDL was associated with apoB. No radioactivity was present in the areas of the gels where apoA-I migrated.

Modification of LDL, HDL, and Fetuin. LDL and HDL were reductively methylated as described by Weisgraber et al. (1978). Methyl-LDL did not inhibit the uptake or degradation of ^{125}I-LDL in cultured porcine fibroblasts (see Results), indicating that it did not bind to the fibroblast LDL receptor. Desialidated fetuin was prepared as described previously (Bachorik et al., 1978). The treatment removed 75% of the sialic acid from fetuin.

Preparation of Porcine Hepatocytes. Hepatocytes were isolated by using an adaptation of the method of Bissell et al. (1973). The livers of Duroc or Yorkshire pigs (2.5–5 kg) were perfused in situ (80–90 mL/min) with 1 L of Ca^{2+}-free modified Krebs' solution, pH 7.4 (Nicholls, 1977), containing EGTA (1×10^{-4} M) and then for 8 min with modified Kreb's solution containing $CaCl_2$ (1×10^{-3} M) and collagenase (0.03% w/v). The liver was disrupted, and the digest was filtered through a coarse nylon mesh and then through a 100-μm nylon mesh. Hepatocytes were harvested and washed 3 times by centrifugation at 50*g*. The cells were cultured in Eagle's minimum essential medium containing penicillin (100 units/mL), streptomycin (100 μg/mL), and 10% FCS. After 4 h, the medium was replaced with fresh 10% FCS-containing medium, and the cells were cultured for an additional 12–16 h before use. The cells attached to the culture plates within 1–2 h, and within 4–6 h, they assumed a flattened, more polygonal appearance. After 16–20 h in culture, over 96% of the cells appeared to be hepatocytes, more than 90% of which excluded 0.02% trypan blue. Electron microscopy revealed characteristic hepatocyte morphology, including large nuclei, numerous cell membrane microvilli, a dense accumulation of mitochondria and rough endoplasmic reticulum, primary and secondary lysosomes, lipid droplets, and numerous glycogen granules, believed to be a sensitive sign of cell integrity (Wanson, 1976). Several metabolic parameters of hepatocyte function were evaluated.

Gluconeogenesis. After 22, 28, or 44 h in culture, the cells were washed 4 times with 3 mL of Eagle's balanced salt solution containing sodium lactate (1×10^{-2} M) and sodium pyruvate (1×10^{-3} M), and incubated with 2.0 mL of the same medium for 4 h. The medium was centrifuged for 10 min at 1000*g* and then applied to a column of Amberlite MB-3 and eluted with H_2O. The eluate was lyophilized and dissolved in 200 μL of H_2O, and glucose was measured enzymatically by using glucose oxidase (Worthington UV glucose reagent set, Worthington Biochemical Corp., Freehold, NJ). [^{14}C]-Glucose (300 Ci/mol, 2×10^5 cpm) added to the medium before ion-exchange chromatography was used to correct for losses during handling. The cells synthesized glucose at a constant rate during the first 2 days in culture [54, 66, and 54 nmol h^{-1} (mg of cell protein)$^{-1}$ after 22, 28, and 44 h in culture, respectively].

Protein, Albumin, and VLDL–Protein Synthesis. After 16 h, the cells were incubated with serum-free medium B (2.0 mL) containing [^3H]leucine (50 μCi/mL) for 2–24 h. The medium was removed and centrifuged at 4 °C for 10 min at 1000*g*, and an aliquot was treated with ice-cold Cl_3CCOOH (final concentration 10% w/v). The precipitate was washed 6 times with 10% Cl_3CCOOH and dissolved in 1 N NaOH, and radioactivity was measured. Porcine albumin (100 μg) was added to a separate aliquot, and the mixture was incubated overnight at 4 °C with monospecific antibody to pig albumin. The immunoprecipitate was washed 4 times with PBS and subjected to $NaDodSO_4$–polyacrylamide gel electropohoresis (7% gels). The gels were cut into 2-mm segments, and the radioactivity was measured. Over 99% of the radioactivity comigrated with authentic porcine albumin and accounted for about 9% of the protein-associated radioactivity in the medium. The cells secreted protein and albumin to the medium at a constant rate for at least 40 h.

VLDL–protein synthesis was measured separately. Cells were cultured initially for 20 h in 10% LPDS-containing medium, after which they were transferred to serum-free medium containing [^3H]leucine (50 μCi/mL) and incubated for 24 h. Unlabeled carrier porcine VLDL was added, and lipoproteins of $d < 1.006$ g/mL were isolated by ultracentrifugation. The floating VLDL layer was washed once by ultracentrifugation and then precipitated with anti-porcine LDL. The immunoprecipitate was washed 6 times with PBS (5 mL), and radioactivity was measured. The cells incorporated over 33 times more [^3H]leucine into VLDL–protein during the 24-h incubation (22 409 cpm/mg of cell protein) than when the incubation was terminated immediately after addition of the radioactive amino acid (673 cpm/mg of cell protein).

Lipoprotein Uptake and Degradation Assays. The medium was replaced with 2.0 mL of fresh medium containing 10% porcine LPDS (7–8 mg/mL serum protein). The cells were incubated with ^{125}I-HDL or ^{125}I-LDL in the presence and absence of the respective unlabeled lipoprotein, as described for each experiment. The medium was then added to 0.5 mL of ice-cold 50% Cl_3CCOOH and allowed to stand for 30 min in an ice bath. The precipitate was removed, and total Cl_3CCOOH-soluble radioactivity was determined as a measure of lipoprotein degradation. In some experiments, a separate aliquot (1.0 mL) of the supernatant was treated with 10 μL of 40% KI and 40 μL of 30% H_2O_2 (Goldstein & Brown, 1974). Unbound iodine was extracted with 4 mL of $CHCl_3$, and radioactivity remaining in the aqueous phase was measured. The cells were washed 5 times with cold PBS containing 0.5% bovine serum albumin and twice with PBS and then dissolved in 1.0 mL of 1 N NaOH for the determination of cellular protein and cell-associated radioactivity. Control incubations were performed without cells, and the measurements were corrected accordingly.

[U-^{14}C]Sucrose Accumulation. Cells were incubated with [U-^{14}C]sucrose (1 μCi/mL, 0.5 μg/mL) under conditions described in the text. The medium was removed, and the cells were washed 12 times with cold PBS and dissolved in 1 M

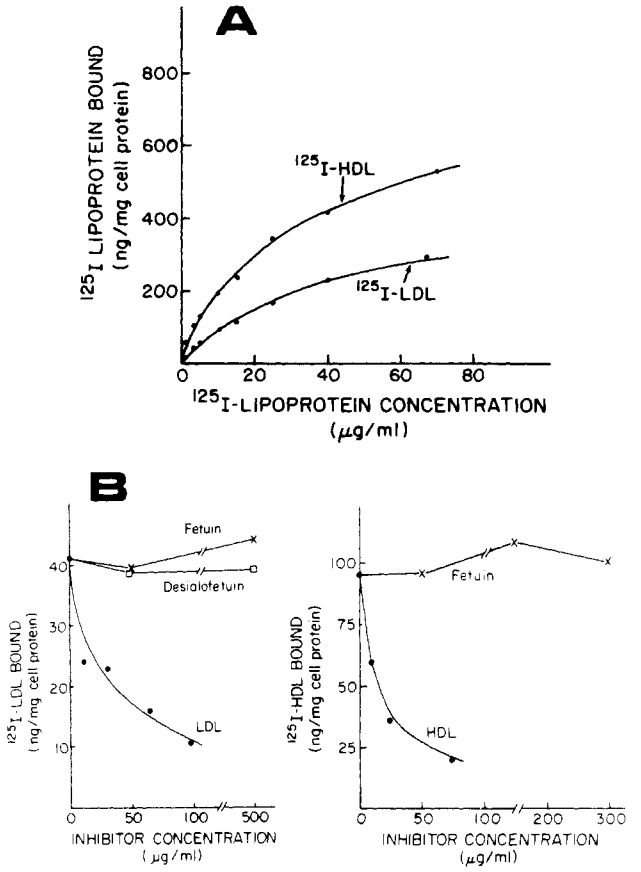

FIGURE 1: Characteristics of porcine ^{125}I-HDL and ^{125}I-LDL binding in cultured porcine hepatocytes at 4 °C. (A) Substrate saturation. After 16 h, cells were transferred to 10% LPDS-containing medium and incubated at 4 °C for 7 h with either ^{125}I-HDL or ^{125}I-LDL at the concentrations indicated. (B) Inhibition by unlabeled substrate and unrelated proteins. Cells were incubated at 4 °C with either ^{125}I-LDL (left panel) or ^{125}I-HDL (right panel) and the indicated concentrations of inhibitor protein. The ^{125}I-labeled lipoprotein concentration used in both cases was 5.0 μg/mL lipoprotein-protein, and the incubation times were 6 (^{125}I-LDL) and 7 h (^{125}I-HDL). Inhibition of the two labeled lipoproteins was tested in different cell preparations.

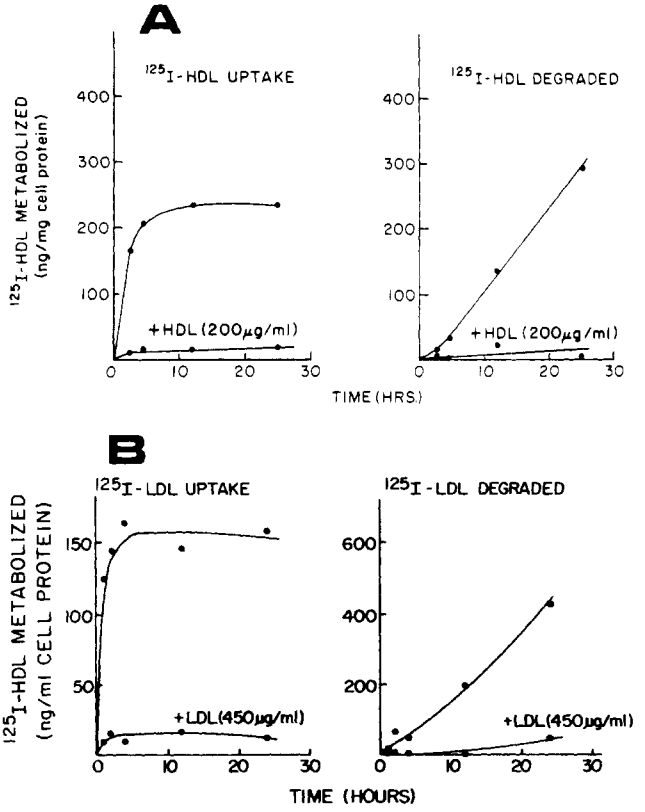

FIGURE 2: Time course of ^{125}I-HDL and ^{125}I-LDL uptake and degradation in cultured porcine hepatocytes at 37 °C. After 16 h, cells were transferred to 10% LPDS-containing medium and incubated with either ^{125}I-HDL (A) or ^{125}I-LDL (B) (5.0 μg/mL lipoprotein-protein) in the presence of the respective unlabeled lipoproteins at the concentrations indicated.

NaOH for measurement of cell protein and cell-associated radioactivity. [U-^{14}C]Sucrose clearance was calculated as described by Miller et al. (1977, 1978).

Results

The association of ^{125}I-HDL and ^{125}I-LDL with hepatocytes was examined at 4 °C. At 4 °C, cell-associated radioactivity was considered to reflect lipoprotein bound to the cell surface (Brown et al., 1976). After 16 h in culture, the cells were washed twice with 3 mL of PBS and transferred to 10% LPDS-containing medium for the binding assays. The binding of both lipoproteins was saturable (Figure 1A). The apparent K_d for each lipoprotein, determined from Scatchard analyses of the data in the figure, was as follows: ^{125}I-HDL, K_d = 2 μg/mL (22 pmol/mL);[2] ^{125}I-LDL, K_d = 10.5 μg/mL (17 pmol/mL). In both cases, binding was inhibited progressively in the presence of increasing concentrations of the respective unlabeled lipoprotein but was unaffected by fetuin, an unrelated protein, when it was added in concentrations corresponding to as much as a 1300-fold molar excess with respect to ^{125}I-LDL or a 115-fold molar excess with respect to ^{125}I-HDL (Figure 1B).

Desialidated fetuin was similarly ineffective as an inhibitor of ^{125}I-LDL binding (Figure 1B), suggesting that the lipoprotein was not bound to the hepatic asialoglycoprotein receptor (Hudgin et al., 1974). Desialidated fetuin was not tested as an inhibitor of ^{125}I-HDL binding. Inasmuch as these assays were performed in the presence of lipoprotein-depleted porcine serum proteins, we concluded that the binding of neither labeled lipoprotein was influenced by unrelated proteins. The substrate-inhibitable binding of both labeled lipoproteins at 4 °C was time dependent. ^{125}I-HDL binding reached equilibrium within 5 h and ^{125}I-LDL binding within about 10 h (data not shown). The results of these experiments suggested that both lipoproteins were bound to limited numbers of specific high affinity sites on the cell.

Uptake and Degradation at 37 °C. Uptake of the two ^{125}I-labeled lipoproteins at 37 °C was considered to represent both surface-bound substrate and that which had been internalized. Cells were incubated at 37 °C with ^{125}I-HDL or ^{125}I-LDL (5 μg/mL lipoprotein-protein) for varying periods. Uptake reached steady state within about 5 h with either substrate (Figure 2). With both substrates, specific degradation was detectable after 2 h of incubation, increased in rate as uptake approached equilibrium, and remained constant (Figure 2) for at least 48 h (not shown).

The comparative clearances of [U-^{14}C]sucrose, ^{125}I-HDL, and ^{125}-LDL were measured to determine whether bulk-phase pinocytosis accounted for an appreciable fraction of lipoprotein uptake and degradation. We assessed the uptake of [U-^{14}C]sucrose and determined whether [U-^{14}C]sucrose was re-

[2] Molar concentrations were calculated from lipoprotein-protein concentrations by assuming molecular weights and protein compositions of 2.5 × 10^6 (25% protein, w/w) and 2.1 × 10^5 (44% protein, w/w) for porcine LDL and HDL, respectively (Davis et al., 1974; Mahley & Weisgraber, 1974; Ikai, 1975; Cox & Tanford, 1968).

Table I: Effect of Lysosomal Inhibitors on ^{125}I-HDL and ^{125}I-LDL Uptake and Degradation in Porcine Hepatocytes

expt no.[a]	lipoprotein	inhibitor	inhibitor concn (M)	cell-associated uptake (ng/mg of cell protein)	degradation (ng/mg of cell protein)	% inhibition
1	^{125}I-HDL[b]	none		240	87	0
		chloroquine	2×10^{-5}	264	37	57
			6×10^{-5}	272	21	76
		leupeptin	2×10^{-5}	245	79	9
			2×10^{-4}	234	73	16
2	^{125}I-HDL[b]	none		391	557	0
		leupeptin	2×10^{-4}	502	425	24
3	^{125}I-LDL[c]	none		101	525	0
		chloroquine	2×10^{-5}	547	387	26
			6×10^{-5}	181	1	>99
		leupeptin	2×10^{-6}	136	431	18
			2×10^{-5}	190	413	21
			2×10^{-4}	202	387	26

[a] The three experiments were performed with different cell preparations. [b] Cells were incubated with ^{125}I-HDL (5 μg/mL) at 37 °C for 10 h (experiment 1) or 24 h (experiment 2) in the presence of the inhibitors. Degradation was calculated from the appearance of total Cl_3CCOOH-soluble radioactivity in the medium. [c] Cells were incubated with ^{125}I-LDL (5 μg/mL) for 12 h at 37 °C in the presence of the inhibitors. Degradation was calculated as indicated above.

leased back to the medium, which would lead to an underestimate of pinocytosis. Two groups of cells were cultured initially for 16 h in 10% FCS. The first group was prelabeled with [U-^{14}C]sucrose (1 μCi/mL) during this period. Both groups were then transferred to 10% LPDS-containing medium. The prelabeled group was maintained in unlabeled medium for 6, 12, and 28 h. Cell-associated radioactivity remained constant during this period, indicating that [U-^{14}C]sucrose was not released by the cells. [U-^{14}C]Sucrose (1 μCi/mL) was added to the second group of cells immediately after transfer to LPDS-containing medium, and the accumulation of radioactivity was determined at various times. The rate of [U-^{14}C]sucrose uptake was constant for at least 29 h and was unaffected by a 100-fold excess of unlabeled sucrose. The calculated rate of bulk-phase pinocytosis was 0.046 μL h^{-1} (mg of cell protein)$^{-1}$.

In two additional groups of cells, the clearances of ^{125}I-HDL and ^{125}I-LDL were measured in parallel with sucrose uptake. The clearance of the two substrates would normally be calculated from the amount of internalized plus degraded lipoprotein. Since uptake included both bound and internalized lipoproteins, however, a calculation based on total cell associated lipoprotein would have overestimated clearance. For this reason, minimum estimates of ^{125}I-HDL and ^{125}I-LDL clearance were made on the basis of only degradation measurements. Degradation was measured by using labeled lipoprotein concentrations of 5 μg/mL. The cells cleared ^{125}I-HDL at a rate corresponding to 2.5 μL of fluid h^{-1} (mg of cell protein)$^{-1}$ and ^{125}I-LDL at the rate of 14.2 μL h^{-1} (mg of cell protein)$^{-1}$. These rates exceeded that of bulk-phase pinocytosis by 54-fold and 309-fold for ^{125}I-HDL and ^{125}I-LDL, respectively, and indicated that bulk-phase pinocytosis accounted for less than 2% of the uptake of either lipoprotein under the conditions of the experiments.

The effects of chloroquine, a general inhibitor of lysosomal hydrolysis, and leupeptin, an inhibitor of cathepsin B (Wingender, 1974), on lipoprotein degradation were examined. ^{125}I-HDL degradation was inhibited by 76% in the presence of 6×10^{-5} M chloroquine and by 16–24% in the presence of leupeptin (2×10^{-4} M) (Table I). Both inhibitors produced a moderate increase in cell-associated ^{125}I-HDL. The inhibitors had similar effects on ^{125}I-LDL degradation. ^{125}I-LDL degradation was inhibited completely by 6×10^{-5} M chloroquine and by 26% in the presence of 2×10^{-4} M leupeptin (Table I). Cell-associated ^{125}I-LDL was increased in the presence of chloroquine and was not affected by leupeptin. The findings suggested that both labeled lipoproteins were degraded primarily in lysosomes.

Production of Free $^{125}I^-$ during Degradation. Deiodinase(s) in hepatocytes catalyze(s) the formation of free $^{125}I^-$ during the catabolism of ^{125}I-labeled lipoproteins (Nakai et al., 1976; Pangburn et al., 1981), and it was considered that direct deiodination of the labeled lipoproteins may produce errors in the degradation measurements. We found that 90% of the Cl_3CCOOH-soluble radioactivity produced from ^{125}I-HDL and about 85% of that produced from ^{125}I-LDL were present in the form of free $^{125}I^-$. The remainder was assumed to represent ^{125}I that remained covalently associated with proteolytic degradation products. As seen in Table I, the degradation of both lipoproteins, measured from the production of total Cl_3CCOOH-soluble radioactivity, was decreased in the presence of lysosomal inhibitors, particularly chloroquine. This indicated that deiodination occurred after the labeled lipoproteins reached the lysosome, but not whether the intact lipoproteins or their degradation products were the primary substrates for deiodination. In order to distinguish these possibilities, we measured degradation in the presence of 3-iodotyrosine, an inhibitor of deiodinase activity, and the Cl_3CCOOH-soluble radioactivity produced was measured after peroxidation and extraction of free $^{125}I^-$. Over 90% of the radioactivity produced from ^{125}I-HDL was in the peroxidation-resistant fraction when 3-iodotyrosine (5×10^{-3} M) was present. In a separate experiment with ^{125}I-LDL, the proportion of Cl_3CCOOH-soluble radioactivity in the peroxidation-resistant fraction increased from 11.7% to 57.2%, or 5-fold, in the presence of 3×10^{-5} M 3-iodotyrosine. The findings in both experiments suggested strongly that free iodide was produced primarily from the proteolytic products rather than directly from the labeled lipoproteins and supported the validity of the practice by which lipoprotein degradation in liver has been determined from the total Cl_3CCOOH-soluble radioactivity produced (Pangburn et al., 1981; Nakai et al., 1976). On the basis of the production of total Cl_3CCOOH-soluble radioactivity, hepatocytes degraded ^{125}I-HDL at the rate of 27 ± 5 ng h^{-1} (mg of cell protein)$^{-1}$ (mean ± SE, n = 7) and ^{125}I-LDL at the rate of 103 ± 37 ng h^{-1} (mg of cell protein)$^{-1}$ (mean ± SE, n = 4) when assayed at substrate concentrations of 5 μg/mL ^{125}I-labeled lipoprotein–protein.

Uptake and Degradation in Pronase-Treated Hepatocytes. Two parallel experiments were performed to evaluate ^{125}I-LDL

Table II: ^{125}I-LDL Binding and Degradation in Pronase-Treated Hepatocytes[a]

pretreatment	specific ^{125}I-LDL binding (ng/mg of cell protein)	^{125}I-LDL degraded[b] (ng/mg of cell protein)
none	48.1	57.1
Pronase		
3 μg/mL	53.0	26.3
5 μg/mL	42.5	24.7

[a] Cells were incubated with ^{125}I-LDL (10 μg/mL) for 10 h at 4 °C in 10% LPDS. Specific binding was calculated as the difference between binding in the presence and absence of unlabeled LDL (300 μg/mL). [b] Cells were incubated with ^{125}I-LDL (15 μg/mL) as described above. The medium was removed, and the cells were transferred to 10% LPDS and incubated without lipoproteins for 11 h at 37 °C. Degradation was measured as described under Methods.

uptake and degradation immediately after treatment of the cells with Pronase. After 16 h in culture, the cells were washed twice with PBS and incubated with Pronase (0, 3, and 5 μg/mL) for 1 h at 37 °C in serum-free medium. The microscopic appearance of the cells remained unchanged. The cells were washed twice with cold PBS and transferred to 10% LPDS, and ^{125}I-LDL binding was measured at 4 °C. Specific binding of ^{125}I-LDL was reduced by 12% in cells treated with 5 μg/mL Pronase; no decrease was observed at the lower Pronase concentration (Table II). In the parallel experiment, the cells were treated with Pronase and then incubated at 4 °C with ^{125}I-LDL as described above. The labeled lipoprotein was removed, the cells were warmed to 37 °C and incubated for 11 h, and the Cl_3CCOOH-soluble radioactivity released to the medium was measured. Degradation was reduced by 54% and 57% in cells treated with 3 and 5 μg/mL Pronase, respectively (Table II). The results of the two experiments indicated that at least the major part of ^{125}I-LDL degradation was susceptible to inactivation by Pronase treatment and that Pronase treatment reduced the degradation of LDL much more than its high-affinity binding.

In similar experiments, Pronase treatment did not affect ^{125}I-HDL binding at 4 °C or degradation when the cells were subsequently warmed to 37 °C (data not shown), suggesting that the binding site for ^{125}I-HDL either was nonprotein in nature or was inaccessible to Pronase.

Effect of HDL on ^{125}I-LDL Binding at 4 °C and on Uptake and Degradation at 37 °C. ^{125}I-LDL binding was measured at 4 °C in the presence of increasing concentrations of unlabeled LDL or apoE-free HDL (Figure 3A). In this figure, the quantities of the two unlabeled lipoproteins are expressed in molar concentrations for comparison.[2] Unlabeled LDL inhibited ^{125}I-LDL binding effectively. HDL also inhibited ^{125}I-LDL binding, but only about two-thirds as effectively as unlabeled LDL. It was, however, effective in low concentration and exerted half-maximal inhibition at about 40 pmol/mL (approximately 4 μg/mL HDL–protein).

^{125}I-LDL uptake and degradation were also measured at 37 °C (Figure 4A). Unlabeled LDL inhibited both the uptake and degradation of ^{125}I-LDL. HDL also inhibited ^{125}I-LDL uptake, and the pattern of inhibition was similar to that observed at 4 °C. HDL maximally inhibited about half the uptake of ^{125}I-LDL and exerted its half-maximal effect at about 100 pmol/mL (about 10 μg/mL HDL–protein). HDL did not inhibit ^{125}I-LDL degradation at all, indicating that only the portion of ^{125}I-LDL uptake was not inhibited by HDL was subsequently degraded. The findings thus suggested the presence of at least two kinds of high-affinity sites that bind ^{125}I-LDL, one of which also recognized HDL.

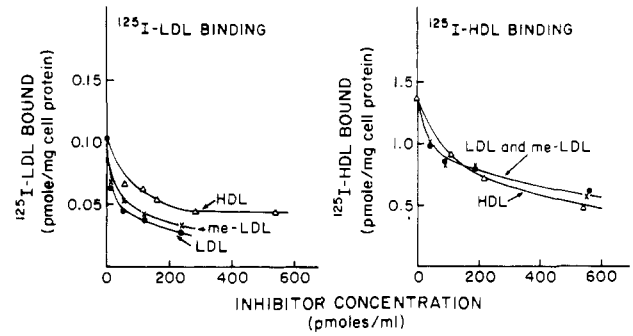

FIGURE 3: Inhibition of binding at 4 °C by native and modified lipoproteins in cultured porcine hepatocytes. After 16 h, cells were transferred to 10% LPDS-containing medium and incubated for 6.5 h at 4 °C with ^{125}I-labeled lipoprotein (5.0 μg/mL lipoprotein–protein). The incubation systems contained HDL, LDL, or methyl-LDL (me-LDL) at the concentrations indicated. One picomole of LDL or HDL was assumed to contain 625 or 93 ng of protein, respectively.[2] (Left panel) ^{125}I-LDL binding; (right panel) ^{125}I-HDL binding; (△) HDL; (●) LDL; (×) me-LDL.

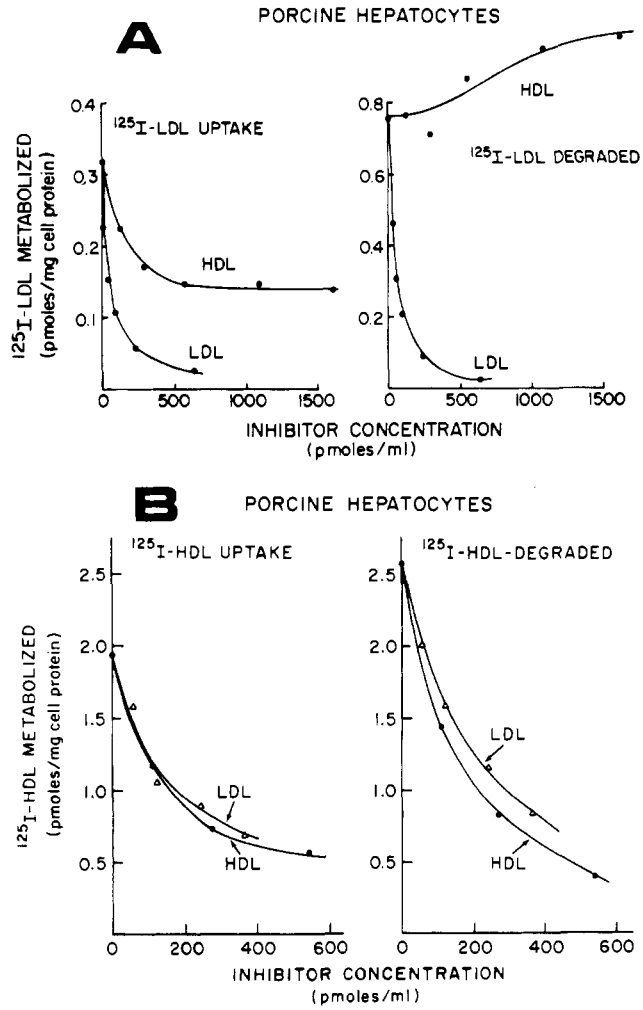

FIGURE 4: Inhibition of ^{125}I-LDL and ^{125}I-HDL uptake and degradation at 37 °C in cultured porcine hepatocytes. After 16 h, hepatocytes were transferred to 10% LPDS and incubated at 37 °C with (A) ^{125}I-LDL (5.0 μg/mL) for 11 h or (B) ^{125}I-HDL (5.0 μg/mL) for 24 h. The incubation systems contained unlabeled LDL or apoE-free HDL at the concentrations indicated. Lipoprotein concentrations are expressed as indicated in the legend to Figure 3.

A similar experiment was performed to determine whether this pattern of ^{125}I-LDL uptake might also occur in porcine

fibroblasts. Unlabeled LDL inhibited both ^{125}I-LDL uptake and degradation, whereas HDL had virtually no effect on either measurement in fibroblasts (not shown). These findings in fibroblasts suggested that unlike hepatocytes, virtually all the ^{125}I-LDL bound to high-affinity sites was susceptible to subsequent degradation.

Effect of LDL on ^{125}I-HDL Binding at 4 °C and on Uptake and Degradation at 37 °C. ^{125}I-HDL binding at 4 °C was inhibited to about the same extent by either unlabeled HDL or LDL (Figure 3B). This pattern of inhibition was also observed at 37 °C (Figure 4B). LDL inhibited both the uptake and degradation of ^{125}I-HDL and was almost as effective an inhibitor as unlabeled HDL. This observation suggested that most ^{125}I-HDL uptake was mediated by a site that recognized both lipoproteins and that this site mediated almost all of the lysosomal degradation of radiolabeled HDL.

Effect of Reductively Methylated Lipoproteins on ^{125}I-LDL and ^{125}I-HDL Binding at 4 °C and Uptake and Degradation at 37 °C. LDL binding to the human fibroblast LDL receptor depends on the presence of intact lysine residues in the lipoprotein, and in our study, methyl-LDL, in which lysine residues were blocked, did not inhibit the uptake or degradation of ^{125}I-LDL in cultured porcine fibroblasts. In heptatocytes, methyl-LDL inhibited ^{125}*i*-LDL binding at 4 °C effectively, but not quite as effectively as native LDL (Figure 3A). The pattern of inhibition of ^{125}I-LDL uptake at 37 °C was similar to that at 4 °C, but the modified lipoprotein reduced ^{125}I-LDL degradation only slightly (Figure 5A).

Methyl-LDL inhibited ^{125}I-HDL binding at 4 °C (Figure 3B) and uptake and degradation at 37 °C (Figure 5B) as effectively as unlabeled LDL. Methyl-HDL also inhibited the uptake and degradation opf ^{125}I-HDL at 37 °C (Figure 5B). The effect of methyl-HDL on ^{125}I-HDL binding at 4 °C was not tested.

These findings suggested that the greatest proportion of the high-affinity binding of ^{125}I-LDL and ^{125}I-HDL in cultured hepatocytes occurred on sites that did not require intact lysine residues in the lipoproteins and that non-lysine-requiring sites mediated little if any ^{125}I-LDL degradation but mediated essentially all the ^{125}I-HDL degradation that occurred under the conditions of the experiments.

Lipoprotein Uptake and Degradation in Lipoprotein-Deprived Cells. LDL receptor activity in peripheral cells is stimulated by cholesterol starvation (Goldstein & Brown, 1977; Brown et al., 1979). In the foregoing experiments, cells were cultured in FCS-containing medium but transferred to LPDS-containing medium for the uptake and degradation assays. Experiments were therefore performed to assess whether the uptake and degradation measurements reflected activities that were present initially in the cells or which emerged during the assays.

After 16 h in 10% FCS, cells were transferred to 10% LPDS and divided into two groups, and the time course of ^{125}I-labeled lipoprotein uptake and degradation was measured in both groups. In the first group, the assays were initiated immediately and in the second group after the cells had remained in LPDS-containing medium for 24 h. The time courses of ^{125}I-LDL uptake and degradation were virtually identical in both groups, and that of ^{125}I-HDL tended to be slightly lower after 24 h in 10% LPDS. These experiments suggested that the uptake and degradation of ^{125}I-LDL and ^{125}I-HDL did not represent activities that emerged during the assays themselves.

Lipoprotein Uptake and Degradation in Cells Preincubated with HDL or LDL. After 16 h in 10% FCS, the cells were divided into three groups. The first was transferred to 10%

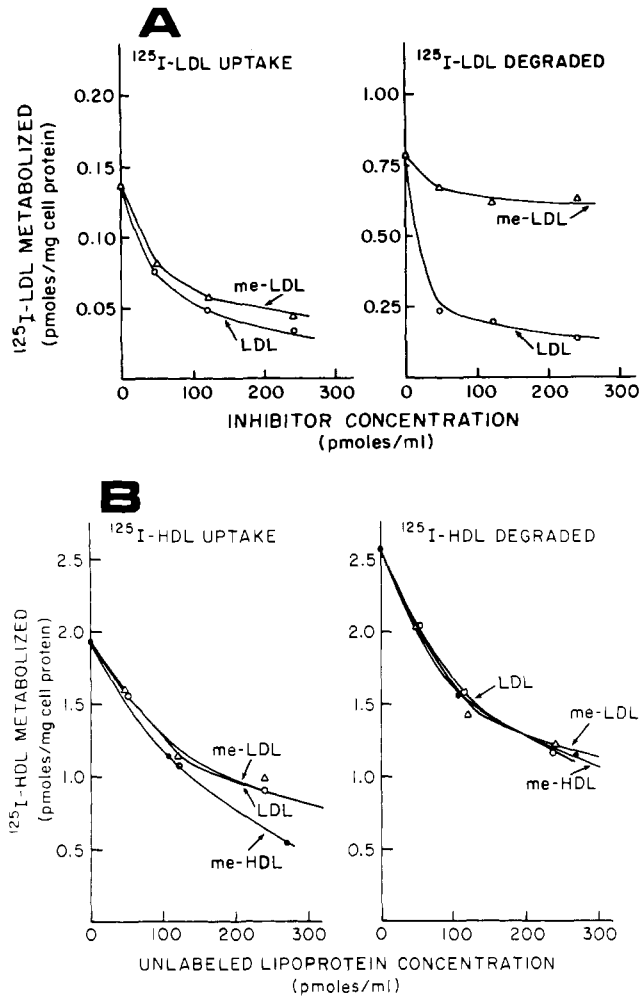

FIGURE 5: Inhibition of ^{125}I-LDL and ^{125}I-HDL uptake and degradation at 37 °C by methyl-LDL and methyl-HDL in cultured porcine hepatocytes. After 16 h, cells were transferred to 10% LPDS and incubated at 37 °C with (A) ^{125}I-LDL (5.0 μg/mL) for 12 or (B) ^{125}I-HDL (5.0 μg/mL) for 24 h. The incubation systems contained unlabeled LDL, methyl-LDL (me-LDL), or methyl-HDL (me-HDL) at the concentrations indicated. The methylated lipoproteins were prepared as indicated under Methods. LDL (○); methyl-LDL (△); methyl-HDL (●).

LPDS, and the uptake and degradation of ^{125}I-LDL were measured immediately. The second group was transferred to 10% LPDS and the third to 10% LPDS containing porcine LDL, and the cells were then preincubated for 24 h at 37 °C. ^{125}I-LDL uptake and degradation were measured at the end of this period. Little change occurred in specific ^{125}I-LDL uptake or degradation during the 24-h preincubation in LPDS (Table III). Preincubation with LDL reduced specific ^{125}I-LDL uptake by about 35% compared with cells that had been deprived of lipoproteins. Specific ^{125}I-LDL degradation was reduced by 68% under these conditions (Table III). Little additional decrease in ^{125}I-LDL uptake and degradation was observed when LDL was also present during the initial 16 h in FCS-containing medium.

In this experiment, another group of cells was grown initially in 10% FCS containing porcine HDL and then preincubated for 24 h in 10% LPDS containing porcine HDL. Preincubation with HDL increased ^{125}I-LDL degradation by almost 60%. Uptake was relatively unaffected under these conditions (Table III). This effect of HDL may have accounted for the slight stimulation of ^{125}I-LDL degradation observed in Figure 4A.

The findings indicated that ^{125}I-LDL degradation was regulated by preincubating the cells with lipoproteins. The

Table III: ^{125}I-LDL Metabolism in Hepatocytes Cultured in the Presence and Absence of Lipoproteins

preincubation conditions[a]		specific[b] ^{125}I-LDL uptake (ng/mg of cell protein)	specific ^{125}I-LDL degradation (ng/mg of cell protein)
0–16 h	16–40 h		
FCS	[c]	69	424
FCS	LPDS	68	471
FCS	LPDS + LDL	44	149
FCS + LDL	LPDS + LDL	34	127
FCS + HDL	LPDS + HDL	58	740

[a] Cells were cultured for the first 16 h in 10% FCS or 10% FCS supplemented as indicated in the first column. The medium was then replaced with 10% LPDS or 10% LPDS supplemented as indicated in the second column, and the cells were maintained for an additional 24 h. FCS provided the cells with 40 μg/mL cholesterol; the added lipoproteins were present in concentrations of 200 μg/mL lipoprotein-protein and provided cholesterol in concentrations of 294 and 82 μg/mL for LDL and HDL, respectively. [b] After preincubation the cells were transferred to 10% LPDS containing ^{125}I-LDL (5 μg/mL) ± unlabeled LDL (300 μg/mL) and incubated for an additional 12 h at 37 °C. Specific uptake and degradation were calculated as the difference between values determined in the absence and presence of the unlabeled lipoprotein. [c] Uptake and degradation assays were initiated at 16 h immediately after transfer to LPDS medium.

most striking observation was the disproportionately greater effect of the preincubation conditions on ^{125}I-LDL degradation than on uptake. These observations support the conclusion that ^{125}I-LDL uptake was mediated by at least two high affinity sites, one of which may not have been suppressed by preincubation with LDL. It is not clear why preincubation with HDL stimulated the degradation of ^{125}I-LDL but not its uptake.

A separate experiment was performed to assess the uptake and degradation of ^{125}I-HDL in cells that were previously exposed to HDL or LDL. ^{125}I-HDL uptake and degradation were not affected in cells that had been preincubated for 24 h with LDL or HDL (200 μg/mL lipoprotein–protein) (not shown).

Discussion

The cells used in this study had morphological and biochemical characteristics associated with functioning hepatocytes. They did not divide, their size, shape, and ultrastructure were characteristic of hepatocytes, and they synthesized glucose, albumin, and VLDL–protein. The cultures contained few, if any, mesenchymal cells.

The high-affinity association of ^{125}I-HDL and ^{125}I-LDL with the cells satisfied several criteria for specific binding. In both cases, binding was time dependent, saturable, and inhibited by the respective unlabeled lipoproteins, but not by unrelated serum proteins. The apparent K_d for binding of either lipoprotein at 4 °C was approximately 2×10^{-8} M. At 37 °C, the high-affinity uptake of either lipoprotein was succeeded by its degradation in lysosomes and occurred at rates that far exceeded those which could be accounted for by bulk-phase pinocytosis. The rate of bulk-phase pinocytosis was 0.046 μL of medium h^{-1} (mg of cell protein)$^{-1}$, a value similar to that [0.038 μL h^{-1} (mg of cell protein)$^{-1}$] previously reported in rat hepatocytes (Ose et al., 1980b). A maximum of less than 2% of ^{125}I-HDL uptake and 0.5% of ^{125}I-LDL uptake would have occurred by this mechanism. These observations indicated that the cells had specific high affinity binding sites for both lipoproteins that mediated their uptake and subsequent lysosomal degradation.

The relation between the uptake and degradation of ^{125}I-LDL was complex. Most of the degraded ^{125}I-LDL was taken up by an LDL-specific mechanism that resembled the peripheral LDL receptor (Goldstein & Brown, 1977; Brown et al., 1979). First, both the uptake and degradation of ^{125}I-LDL were suppressed when cells were preincubated with LDL, and second, degradation was reduced in Pronase-treated cells but was affected very little by apoE-free HDL or methyl-LDL, neither of which is recognized by the LDL receptor (Mahley et al., 1977b; Innerarity & Mahley, 1978; Pitas et al., 1979; Innerarity et al., 1980; Mahley & Innerarity, 1977; Weisgraber & Mahley, 1980). The Ca^{2+} dependence of ^{125}I-LDL uptake and degradation could not be examined satisfactorily because the cells detached from the culture plates in Ca^{2+}-free medium or in the presence of EDTA. These characteristics of ^{125}I-LDL degradation were similar to those recently reported by Pangburn et al. (1981). The relation between degradation and uptake was not reported for most of their experiments, but their findings also suggested the existence in hepatocytes of a receptor similar to the peripheral LDL receptor.

The LDL-specific site did not account for all the high-affinity uptake of ^{125}I-LDL, however, as revealed by the disproportionate effects of various inhibitors on ^{125}I-LDL uptake and degradation. Methyl-LDL and apoE-free HDL both inhibited a substantial proportion of ^{125}I-LDL uptake, but neither inhibitor affected degradation very much. It was noted that methyl-LDL inhibited a greater proportion of ^{125}I-LDL uptake than HDL did, but the significance of this finding is not clear. One possibility is that the modified lipoprotein was partially demethylated by the cells during the assays, and the regenerated LDL contributed to the inhibition. This explanation is unlikely, however, because methyl-LDL inhibited uptake to a greater extent than HDL, even at 4 °C. Regardless of the quantitative difference between the two inhibitors, the inhibition patterns suggested that half or less of the total labeled LDL bound to high-affinity sites was subsequently degraded. This conclusion was supported further by several other observations. Treatment of the cells with Pronase under conditions known to inactivate the LDL receptor (Goldstein & Brown, 1974) reduced ^{125}I-LDL degradation to a 5-fold greater extent than it reduced uptake. Preincubation with LDL suppressed ^{125}I-LDL degradation twice as much as it reduced uptake. Finally, preincubation with HDL stimulated LDL degradation but did not affect LDL uptake. It is not clear why at least some increase in LDL uptake was not observed under these conditions. Nonetheless, the findings suggest that while an LDL receptor like site mediated most ^{125}I-LDL degradation, it accounted for a relatively small proportion of ^{125}I-LDL binding. A large amount of ^{125}I-LDL was bound to a different kind of high-affinity site(s) that (1) recognized both LDL and apoE-free HDL, (2) did not require intact lysine residues in the lipoprotein for recognition, and (3) was relatively insensitive to inactivation by Pronase. We previously reported a specific high affinity lipoprotein binding site with these properties in microsomes isolated from pig liver and other tissues (Bachorik et al., 1976) and from isolated porcine liver plasma membranes (Bachorik et al., 1978, 1981). The present work suggests that the lipoprotein binding site may exist in intact hepatocytes also. The two sites were not clearly distinguished in the Scatchard analysis from which the apparent K_d for ^{125}I-LDL binding was determined, suggesting they had similar affinities for LDL.

^{125}I-HDL uptake and degradation were apparently mediated by a site that resembled the lipoprotein binding site in that binding and degradation were insensitive to Pronase and did not require intact lysine residues in the lipoprotein. This site was not regulated by preincubation with lipoproteins. The most striking finding, however, was that LDL inhibited both the uptake and degradation of ^{125}I-HDL, and did so almost as effectively as unlabled HDL. The ^{125}I-HDL site, therefore, appeared to recognize HDL and LDL, even though it did not recognize other serum proteins. The similarity in the properties and lipoprotein specificity of Pronase-insensitive ^{125}I-LDL uptake and ^{125}I-HDL uptake suggests that the same high-affinity site was responsible for both. The HDL preparations used for the study contained no apoB or apoE, and the LDL contained no detectable apoA-I, and if these apoproteins participate in the association of either HDL or LDL with the site, recognition would probably involve structurally homologous regions of the apoproteins rather than the entire apoprotein molecules. Phosphatidylcholine, and to a lesser extent sphingomyelin, inhibits ^{125}I-LDL binding in isolated pig liver membranes (Bachorik et al., 1978), raising the possibility that phospholipids, which are common components of HDL and LDL, may participate in the binding reaction. If so, it is unlikely that the association simply represents the uptake of lipoprotein phospholipids by phospholipid-deficient areas of the cell membrane, because the ability of the cells to bind ^{125}I-HDL was not reduced when the cells were preincubated with HDL or LDL. Regardless of the binding mechanism, the pattern of inhibition by LDL suggests that the site accounted for almost all the ^{125}I-HDL uptake and lysosomal proteolysis that occurred in the experiments.

The observations are consistent with the following interpretation. Cultured porcine hepatocytes exhibited at least two distinct high affinity sites for lipoproteins. The first had properties similar to those of the fibroblast LDL receptor and mediated LDL degradation. The second site recognized apoE-free HDL and LDL and mediated the degradation of HDL but probably not of LDL.

Evidence for high-affinity HDL or LDL uptake has been presented by others in freshly suspended rat liver parenchymal and nonparenchymal cells (Van Berkel et al., 1980; Ose et al., 1980; Ose et al., 1979; Nakai et al., 1976) and in cultured rabbit and pig hepatocytes (Soltys & Portman, 1979; Pangburn et al., 1981), and a binding site with properties of the peripheral LDL receptor has been detected in liver microsomes from several species (Kovanen et al., 1979, 1981; Kita et al., 1981; Hui et al., 1981). There is some suggestion in several of these studies of the presence of a site(s) similar to the lipoprotein binding site as well. HDL effectively inhibited ^{125}I-LDL binding in rat (Ose et al., 1980) and rabbit Soltys & Portman, 1979) hepatocytes. Nakai et al. (1976) found that rat ^{125}I-HDL uptake was inhibited by LDL in rat hepatocytes, and conversion of their data to molar concentrations suggests that LDL was at least as effective as unlabeled HDL. Ose et al. (1981) and Ose et al. (1980) made a similar observation, but in none of the studies was the influence of LDL on ^{125}I-HDL degradation investigated. While these studies are suggestive, they cannot be interpreted unequivocally, since apoE, which constitutes about 10% of the protein in rat HDL (Quarfordt et al., 1978), was not removed from the HDL preparations, and the two lipoproteins may have competed for a site (LDL receptor) that recognized apoB and apoE.

Kita et al. (1981) have recently found in isolated rabbit liver microsomes that ^{125}I-LDL was bound to at least two kinds of high-affinity sites, one of which resembled the peripheral LDL receptor. ^{125}I-LDL binding to the second site occurred in the presence of EDTA and was inhibited by HDL or methyl-LDL, as well as by unlabeled LDL. Apparently, at least part of the ^{125}I-LDL was bound to a site that also recognized HDL.

The HDL site in pig hepatocytes mediated the lysosomal degradation of ^{125}I-HDL, but it is presently unknown whether this activity represents its major physiological function. Assuming that the site does participate in in vivo HDL catabolism, we can only conjecture the consequence of its recognition of both HDL and LDL. Although LDL and unlabeled HDL in equimolar concentrations inhibited ^{125}I-HDL uptake and degradation to about the same extent in cultured hepatocytes, the molar concentrations of HDL in pigs exceed those of LDL by about 7-fold in adult animals (Knipping et al., 1975; Janado et al., 1966) and by about 20-fold in young animals (Calvert & Scott, 1974). With the assumption the hepatic parenchymal cells are exposed to circulating levels of the two lipoproteins in vivo, little direct interference by LDL would be expected, and the site might manifest a functional specificity for HDL despite its ability to recognize both lipoproteins. Studies are currently in progess to examine the potential consequences of HDL uptake with respect to cholesterol and apoprotein biosynthesis in cultured hepatocytes.

Acknowledgments

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