Receptor-mediated uptake of low density lipoprotein stimulates bile acid synthesis by cultured rat hepatocytes

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Abstract The cellular mechanisms responsible for the lipoprotein-mediated stimulation of bile acid synthesis in cultured rat hepatocytes were investigated. Adding 280 μg/ml of cholesterol in the form of human or rat low density lipoprotein (LDL) to the culture medium increased bile acid synthesis by 1.8- and 1.6-fold, respectively. As a result of the uptake of LDL, the synthesis of [^{14}C]cholesterol from [2-^{14}C]acetate was decreased and cellular cholesteryl ester mass was increased. Further studies demonstrated that rat apoE-free LDL and apoE-rich high density lipoprotein (HDL) both stimulated bile acid synthesis 1.5-fold, as well as inhibited the formation of [^{14}C]cholesterol from [2-^{14}C]acetate. Reductive methylation of LDL blocked the inhibition of cholesterol synthesis, as well as the stimulation of bile acid synthesis, suggesting that these processes require receptor-mediated uptake. To identify the receptors responsible, competitive binding studies using ^{125}I-labeled apoE-free LDL and ^{125}I-labeled apoE-rich HDL were performed. Both apoE-free LDL and apoE-rich HDL displayed an equal ability to compete for binding of the other, suggesting that a receptor or a group of receptors that recognizes both apolipoproteins is involved. Additional studies show that hepatocytes from cholestyramine-treated rats displayed 2.2- and 3.4-fold increases in the binding of apoE-free LDL and apoE-rich HDL, respectively. These data show for the first time that receptor-mediated uptake of LDL by the liver is intimately linked to processes activating bile acid synthesis.—**Junker, L. H., and R. A. Davis.** Receptor-mediated uptake of low density lipoprotein stimulates bile acid synthesis by cultured rat hepatocytes. *J. Lipid Res.* 1989. **30:** 1933–1941.

Supplementary key words cholesterol synthesis • apoE-free LDL • apoE-rich HDL • competitive binding studies • receptors

Metabolism of cholesterol to the bile acids is liver-specific process, and is one of the primary mechanisms involved in maintaining whole body cholesterol homeostasis. When the contribution of this pathway was estimated in rats, it was found that 80–94% of an injected dose of radioactive cholesterol was excreted as biliary cholesterol and bile acids (1). The bile acid synthetic pathway is controlled by cholesterol 7α-hydroxylase (EC 1.14.13.7), a cytochrome P-450 enzyme localized in the endoplasmic reticulum (2–4). The substrate for bile acid synthesis can be derived from both endogenous (newly synthesized) and exogenous (lipoprotein) cholesterol. Several reports suggest that the preferred substrate for bile acid synthesis is newly synthesized cholesterol (5, 6). Radioisotope pulse studies in humans using radiolabeled cholesterol show that HDL unesterified cholesterol can be delivered for both acid synthesis and biliary cholesterol (7, 8). Whether these results reflect a rapid movement of a small mass of cholesterol or are the result of a quantitatively significant pathway remains unknown. Circulating lipoproteins are recognized by the liver and are removed by both receptor-dependent and -independent mechanisms. Receptor-mediated uptake of HDL has been shown to stimulate bile acid synthesis in cultured hepatocytes prepared from chick embryos (9) and rats (10). Recently, our laboratory demonstrated that rat apoE-rich HDL stimulates bile acid synthesis twofold in cultured rat hepatocytes (11). In contrast, when apoE-rich HDL was methylated, it was no longer capable of stimulating bile acid synthesis, suggesting that receptor-mediated uptake was required. In addition, human HDL that contained little, if any, apoE did not stimulate bile acid synthesis (11).

Receptor-dependent uptake of LDL accounts for 60–90% of its removal from the circulation in different species (12–15). While most cells express LDL receptors, quantitatively the liver is the most important site of uptake (15, 16). It is well established that receptor-mediated uptake of LDL leads to cholesteryl ester accumulation and down-regulation of cholesterol synthesis in hepatocytes (17, 18) and HepG2

Abbreviations: BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; EDTA, ethylenediamine-tetraacetic acid, tetrasodium salt; GLC, gas–liquid chromatography; HDL, high density lipoprotein; LDL, low density lipoprotein; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TCA, trichloroacetic acid; TLC, thin-layer chromatography.

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cells (19, 20). However, it is not known what effect, if any, receptor-mediated uptake of LDL has on bile acid synthesis. In this study the effect of apoB- and apoE-containing lipoproteins on bile acid synthesis was examined in cultured rat hepatocytes. The results show that apoE-free LDL is taken up by hepatocytes via a receptor-mediated process and stimulates bile acid synthesis. Furthermore, common receptor(s) appear to be responsible for binding of both apoE and apoB in cultured rat hepatocytes.

MATERIALS AND METHODS

Reagents for tissue culture and chemical supplies were obtained from sources as previously described (11).

Lipoprotein isolation and characterization

Blood was obtained from male Sprague-Dawley rats weighing 400–500 g. In some experiments rats were fasted for 48 h in order to increase the fraction of HDL that was apoE-rich (11). Lipoproteins were isolated by ultracentrifugation using KBr as described (21). The following densities were used for isolation of lipoproteins: LDL d 1.03–1.07 g/ml and HDL d 1.07–1.21 g/ml. Lipoproteins were extensively dialyzed against 2 mM sodium phosphate, 0.5 mM EDTA, 0.1 mg/ml sodium azide (pH 7.4). The LDL fraction was further purified free of apoE using a rabbit polyclonal antibody against rat apoE. An IgG fraction was prepared from the apoE antiserum using a 0–50% ammonium sulfate precipitation. This fraction was dialyzed against the sodium phosphate buffer (described above) and incubated with the LDL fraction (15 mg IgG/mg LDL protein) for 2–4 h at 4°C. The solution was then adjusted to d 1.07 g/ml using solid KBr, and LDL was reisolated by ultracentrifugation. HDL was separated by heparin-Sepharose chromatography, yielding an apoE-rich HDL fraction as described (11). LDL was reductively methylated as described (22). The apolipoprotein composition of the lipoprotein fractions was characterized by SDS-PAGE (23). Cholesterol and cholesteryl ester content was determined by GLC (24).

Iodination of lipoproteins

Lipoprotein fractions isolated as described above were extensively dialyzed against 150 mM NaCl, 0.24 mM EDTA, 0.1 mg/ml sodium azide, pH 7.4 (buffer A) and iodinated with carrier free $Na^{125}I$ using the iodine monochloride method as modified (25). Greater than 90% of the total radioactivity was TCA-precipitable, while less than 5% of the total radioactivity was extractable with $CHCl_3$–MeOH 2:1. Protein concentration was determined by the method of Lowry et al. (26). The specific activity of the lipoprotein fractions was 200–500 cpm/ng. In some experiments apoE-rich HDL was iodinated by the Bolton Hunter method (27) using 1 mCi carrier free $Na^{125}I$/mg protein. The specific activity of the apoE-rich HDL was 300–500 cpm/ng protein with greater than 90% of the total radioactivity being TCA-precipitable.

Preparation of hepatocytes

Hepatocytes from male Sprague-Dawley rats weighing 200–300 g were prepared as described in detail (28). In most cases, rats were fed water and chow ad libitum. In other experiments, rats were fed chow containing 4% cholestyramine for 2 weeks prior to the cell preparation. Cells were plated for 4 h at 37°C in Dulbecco's Modified Eagle's medium (DMEM: Gibco, medium #78-5433 without arginine and pyruvate) containing 20% calf serum and 10 mM glucose. Studies on the bile acid and lipid synthetic rate were performed in 60-mm dishes, while ^{125}I-labeled lipoprotein binding studies were done in 35-mm dishes.

Bile acid and lipid synthetic studies

Cells were prepared as described above and plated for 4 h in 60-mm culture dishes (3 ml at 1.3×10^6 cells/ml) in DMEM containing 20% calf serum and 10 mM glucose. After 4 h the media was changed to serum-free DMEM containing lipoproteins at the concentration indicated in the legends. Incubations were carried out for 15 h at 37°C and cells pulsed for 1 h with 5 μCi [2-^{14}C]acetate/plate. Cell pellets were extracted with $CHCl_3$–MeOH 2:1, free and esterified cholesterol were determined by GLC, and the incorporation of [2-^{14}C]acetate into cellular lipids was determined as previously described (24). The media mass of cholic and β-muricholic acids was determined by GLC using electron capture detection of derivatized bile acids as described in detail (29).

Lipoprotein binding studies

Hepatocytes were prepared as described above and plated in 35-mm dishes using 2 ml of 8×10^5 cells/ml. Binding studies were performed essentially as described (25). Cells were plated for 4 h at 37°C, after which the culture medium was changed to serum-free DMEM and cooled at 4°C for 1 h. ^{125}I-Labeled lipoprotein (10 μg) was then added together with the indicated amount of unlabeled competitor. After incubating cell dishes for 2 h at 4°C, cells were washed five times in buffer containing 150 μM NaCl, 50 mM Tris-HCl, 2 mg/ml fatty acid-free BSA, pH 7.4. Cells were harvested, centrifuged for 20 min at 2000 RPM and the cell pellet was solubilized in 1 ml of 1 M NaOH. Cell-associated ^{125}I was quantitated using a gamma counter and results were expressed as cpm bound/mg cell protein.

RESULTS

Regulation of hepatocyte cholesterol and bile acid synthesis by LDL

To examine the ability of LDL to stimulate bile acid synthesis, rat and human LDL were added to cultured rat

TABLE 1. Effect of lipoproteins on mass and the incorporation of [2-^{14}C]acetate into cellular free cholesterol (FC) and cholesteryl esters (CE)

	^{14}C Incorporation		Cellular Mass	
	FC	(CE)/(CE + FC)	FC	CE
	cpm/mg cell protein	*%*	*μg/mg cell protein*	
Control	6008 ± 689	9.6 ± 0.4	15.0 ± 0.8	5.2 ± 0.7
Rat LDL	2240 ± 240*	26.8 ± 1.1*	16.1 ± 1.2	9.4 ± 1.6*
Human LDL	3962 ± 100*	12.5 ± 0.5*	16.4 ± 0.4	8.5 ± 1.6*

Cultured rat hepatocytes were prepared as described in Methods. Incubations were performed in serum-free DMEM containing rat LDL (270 μg protein/ml media) and human LDL (200 μg protein/ml media) added at equal cholesterol concentrations (280 μg/ml). The incorporation of [^{14}C]acetate into [^{14}C]cholesterol was determined by separating lipids by TLC and assaying the radioactivity in individual lipids. Free and total cholesterol concentrations were determined by GLC (24). Values shown are means ± SD for n = three cell dishes from the same preparation of cells.
*Different from control incubations at $P < 0.05$; significance was determined by Student's *t*-test.

hepatocytes and the effects on bile acid and cholesterol synthesis were determined. Both rat and human LDL inhibited the synthesis of [^{14}C]cholesterol and increased the percent of [^{14}C]cholesterol esterified (**Table 1**). Furthermore, both human and rat LDL fractions resulted in an accumulation of cellular cholesterol esters, whereas the cellular concentration of free cholesterol was not significantly affected. This experiment was repeated two additional times using different preparations of hepatocytes and lipoproteins. Similar results were obtained in all experiments.

The effects of LDL on bile acid synthesis were then examined. Since cellular concentrations of bile acids remain constant, the amount of cholic and β-muricholic acids that is secreted into the culture medium is an accurate measure of bile acid synthesis (29). In overnight incubations both rat and human LDL stimulated total bile acid synthesis (measured as the sum of cholic and β-muricholic acids) by 2.1- and 1.7-fold, respectively (**Fig. 1**). When bile acid synthetic rates were measured using two additional preparations of hepatocytes and lipoproteins, a similar stimulation was observed. These data clearly demonstrate the ability of both rat and human LDL to deliver cholesterol to the hepatocyte and, as a result, de novo cholesterol synthesis is decreased, cholesterol accumulates as cholesteryl esters, and bile acid synthesis is stimulated.

Since both large molecular weight apoB ($apoB_L$) and apoE are recognized by the LDL receptor (30), it is possible that either one of these apolipoproteins is responsible for the recognition and uptake of LDL by cultured rat hepatocytes. In contrast, human LDL contains essentially only apoB-100, suggesting that this apolipoprotein may be sufficient for directing the uptake of LDL and subsequent stimulation of bile acid synthesis. To investigate whether homologous apoB is sufficient to stimulate bile acid synthesis, a fraction of rat LDL was prepared which contained no detectable apoE. Using an apoE-specific IgG, followed by an ultracentrifugation step to remove the apoE–IgG complex from the lipoprotein fraction, an LDL fraction with no detectable apoE was obtained (**Fig. 2**). The relative abilities of apoE-free LDL and apoE-rich HDL to affect cholesterol metabolism in cultured hepatocytes were then examined. When added at equal lipoprotein cholesterol concentrations, apoE-free LDL and apoE-rich HDL inhibited de novo cholesterol synthesis by 49% and 33%, respectively (**Fig. 3A**). Moreover, both apoE-free LDL and apoE-rich HDL stimulated bile acid synthesis 1.5-fold (Fig. 3B). When this experiment was repeated using two additional preparations of hepatocytes and lipoproteins, similar results were obtained. These data show that lipoproteins containing either apoB or apoE can deliver cholesterol to hepatocytes and bile acid synthesis is stimulated.

We also examined whether the results obtained with apoE-free LDL could be influenced by reductive methylation, a process that is known to block recognition by the LDL receptor (22). Reductive methylation of apoE-free

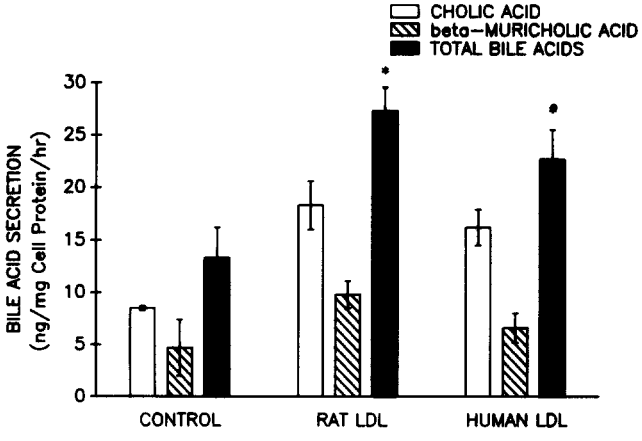

Fig. 1. Effect of rat and human LDL on bile acid secretion in cultured rat hepatocytes. Incubations were performed as described in Table 1 and bile acid synthesis was determined by GLC as described (29). Values shown are mean ± SD for n = 3 cell plates from the same hepatocyte preparation. Significance was determined by Student's *t*-test and asterisks denote significant differences from control incubations at $P < 0.05$.

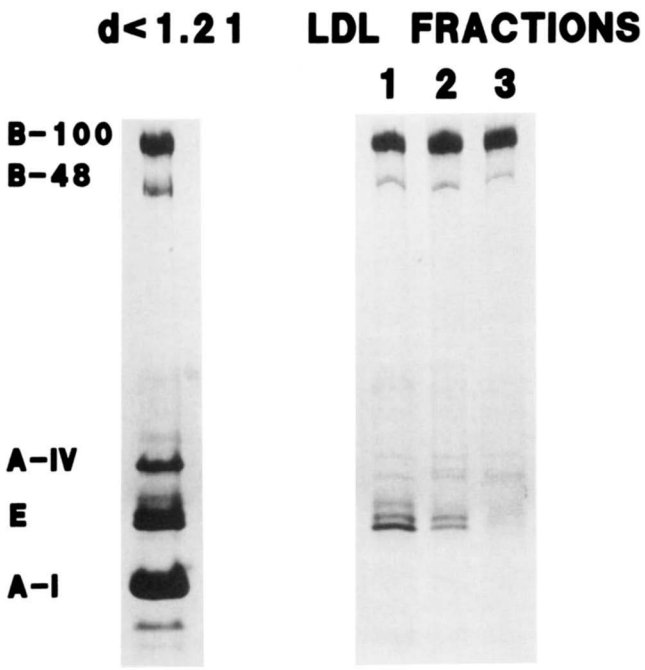

Fig. 2. Purification of rat apoE-free LDL. Rat LDL isolated by ultracentrifugation (d 1.03–1.07 g/ml) was incubated with an IgG prepared against apoE. The LDL was reisolated by ultracentrifugal flotation at d 1.07 g/ml, and subjected to SDS-PAGE as described (23). Left lane, d < 1.21 g/ml lipoprotein standard. LDL fractions are as follows: lane 1, original LDL isolated by ultracentrifugation; lane 2, first cycle of antibody incubation and LDL reisolation; lane 3, second cycle of LDL purification.

LDL blocked both the inhibition of [^{14}C]cholesterol synthesis (Fig. 3A) and stimulation of bile acid synthesis (Fig. 3B) in this experiment. This was observed again using a different preparation of hepatocytes and methylated LDL. These data suggest that receptor-mediated uptake of LDL is required for it to stimulate the secretion of bile acids.

Characterization of lipoprotein binding

To characterize the receptors responsible for the uptake of LDL and apoE-rich HDL, the binding of ^{125}I-labeled rat lipoproteins to intact cultured rat hepatocytes was examined. The ability of unlabeled lipoprotein (either apoE-free LDL or apoE-rich HDL) to compete for the binding of 10 μg of ^{125}I-labeled apoE-free LDL was first determined. Approximately half of the total binding of ^{125}I-labeled apoE-free LDL was competed for by either unlabeled apoE-free LDL or apoE-rich HDL (**Fig. 4A**). The specific binding constants were calculated as the difference between binding in the presence and absence of an excess (400 μg/ml) of each unlabeled competitor. The specific ^{125}I-labeled apoE-free LDL binding competed for by apoE-free LDL was calculated to be 15.5 ± 2.3 ng LDL protein/mg cell protein, which was 57.6 ± 8.7% of the total cell-associated radioactivity. The specific binding constant using apoE-rich HDL was calculated to be 14.4 ± 1.0 ng LDL protein/mg cell protein (53.7 ± 3.7% of the total binding). These data show that both unlabeled apoE-rich HDL and apoE-free LDL competed for the binding of ^{125}I-labeled apoE-free LDL to the same extent. Increasing the concentration of either unlabeled apoE-free LDL or apoE-rich HDL to 1 mg (100-fold excess) did not further decrease the binding of ^{125}I-labeled apoE-free LDL. These data suggest that maximum competition had been achieved (data not shown). Nonspecific binding that could not be competed for by lipoprotein amounted to about half of total binding. This high background has been previously reported to be present in rat liver membranes and may be a characteristic of liver (31). The specific binding of human LDL to site 1 in rat hepatocytes cultured for 20 h was 14.4 ± 4.6 ng LDL protein/mg cell protein (Table 1 of Ref. 32). This apparent specific binding capacity is in good agreement with the specific binding constants we obtained using either

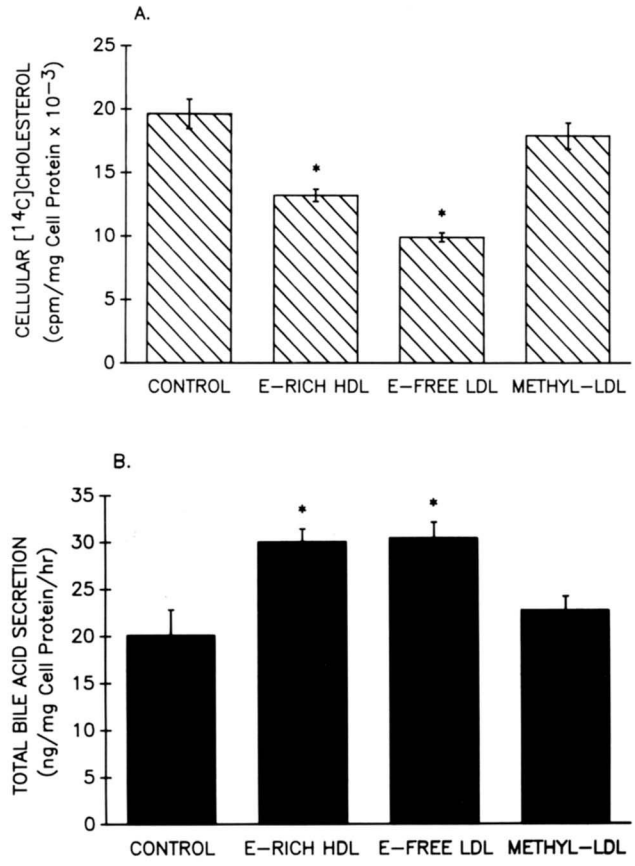

Fig. 3. Effect of lipoproteins on cholesterol synthesis (A) and bile acid secretion (B) by cultured rat hepatocytes. Rat apoE-rich HDL (500 μg protein/ml), rat apoE-free LDL and methylated rat LDL (270 μg protein/ml) were added at equal cholesterol concentrations (200 μg/ml media) and incubations were performed as described in Table 1. The incorporation of [^{14}C]acetate into [^{14}C]cholesterol and bile acid synthesis were determined as described in Table 1. Values shown are mean ± SD for n = 3 cell plates from the same hepatocyte preparation. Significance was determined by Student's *t*-test and asterisks denote significant differences, $P < 0.05$.

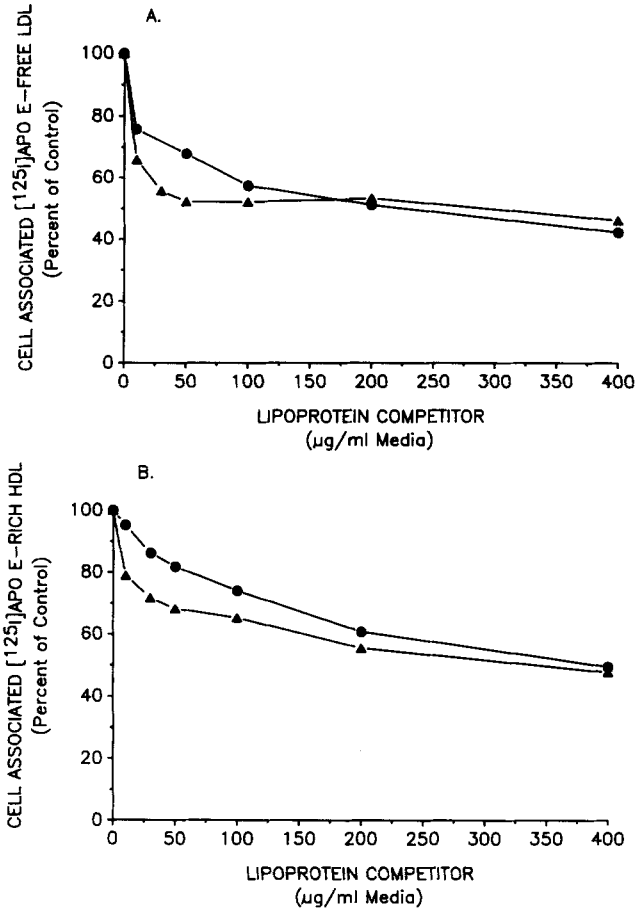

Fig. 4. Competition of the binding of rat ^{125}I-labeled apoE-free LDL (A) and rat ^{125}I-labeled apoE-rich HDL (B) to control rat hepatocytes by unlabeled rat apoE-free LDL (●) and rat apoE-rich HDL (▲). Binding studies were performed at 4°C using 10 μg ^{125}I-labeled lipoprotein and increasing concentrations of unlabeled competitors. The 100% control values for binding of 10 μg ^{125}I-labeled lipoproteins were 26.9 ± 3.8 (ng protein/mg cell protein) for the rat apoE-free LDL and 124 ± 26 for the rat apoE-rich HDL. Each point represents the mean ± SD from duplicate dishes of n = 3 binding studies using three different preparations of hepatocytes.

unlabeled apoE-free LDL (15.5 ± 2.3 ng LDL protein/mg cell protein) or apoE-rich HDL (14.4 ± 1.0 ng/mg cell protein) as competitors of ^{125}I-labeled apoE-free LDL binding. Recently, Salter et al. (32) reported the existence of two high affinity binding sites on cultured rat hepatocytes. Additional characterization of the binding sites showed that site 1 requires Ca^{2+}, whereas site 2 is only partially Ca^{2+}-dependent (33). We have not examined the requirement for Ca^{2+} in our binding studies.

Binding studies were also performed using 10 μg of ^{125}I-labeled apoE-rich HDL (Fig. 4B). ApoE-free LDL inhibited the binding of ^{125}I-labeled apoE-rich HDL to the surface of rat hepatocytes to the same degree as that obtained using unlabeled apoE-rich HDL. Addition of a 100-fold excess of unlabeled apoE-rich HDL (1 mg) did not significantly increase the competition of cell-associated radioactivity (data not shown). The specific binding of the ^{125}I-labeled apoE-rich HDL competed for by apoE-free LDL was calculated to be 62.4 ± 9.5 ng HDL protein/mg cell protein, which accounted for 50.3 ± 7.7% of the total cell-associated radioactivity. The specific binding constant describing the binding of apoE-rich HDL was 64.6 ± 7.1 ng HDL protein/mg cell protein (52.1 ± 5.7% of the total binding). These data suggest that the high affinity ("competitive") receptors for rat apoB-LDL and apoE-rich HDL in cultured rat hepatocytes are common.

Effect of cholestyramine feeding on the binding of lipoproteins to cultured rat hepatocytes

Treatment of animals with the bile acid sequestrent cholestyramine has been shown to induce expression of hepatic LDL receptors in different species (34–36). We examined whether cultured rat hepatocytes obtained from rats fed cholestyramine would bind more ^{125}I-labeled lipoproteins. Hepatocytes were prepared from control and cholestyramine-treated rats and binding studies were performed using identical preparations of ^{125}I-labeled apoE-free LDL and ^{125}I-labeled apoE-rich HDL. The specific binding constant of apoE-free LDL was determined by binding that could be displaced by a 100-fold excess (1 mg) of homologous unlabeled lipoprotein, while total binding was determined in the presence of 10 μg of ^{125}I-labeled lipoprotein alone. Essentially all of the increase in total binding in the cells from the cholestyramine-treated animals could be accounted for by a 2.2-fold higher specific binding of apoE-free LDL (**Fig. 5**). There was a similar increase in the specific binding of constant apoE-rich HDL to hepatocytes obtained from cholestyramine-fed rats (3.4-fold increase; Fig. 5). When different preparations of hepatocytes were used, similar results were obtained for the specific binding constants of apoE-rich HDL and apoE-free LDL. These data show that cholestyramine feeding specifically increased the "competitive" binding. Moreover, the ability of rat hepatocytes in culture to express an increase in specific binding of both apoB- and apoE-containing lipoproteins demonstrates that this experimental model accurately reflects changes induced in vivo.

DISCUSSION

The results of this study demonstrate that, in cultured rat hepatocytes, receptor-mediated uptake of LDL leads to increased cellular concentrations of cholesteryl esters, down-regulation of cholesterol biosynthesis, and a stimulation of bile acid synthesis. Additional evidence is presented showing that apoB is sufficient to direct the hepatic uptake of LDL leading to a stimulation of bile acid synthesis. Since the stimulation of bile acid synthesis by LDL is blocked by reductive methylation, it is likely that receptor-

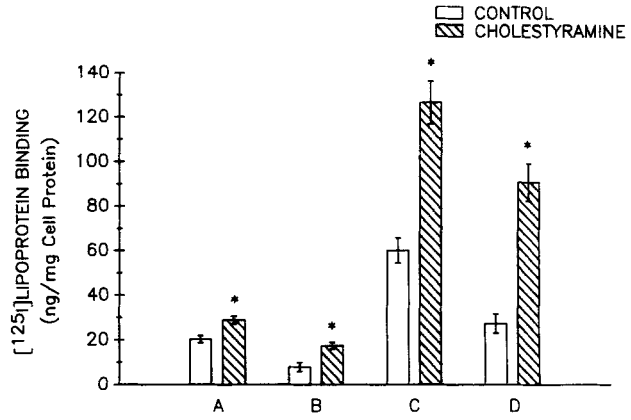

Fig. 5. Effect of cholestyramine treatment on the binding of rat ^{125}I-labeled apoE-free LDL and rat ^{125}I-labeled apoE-rich HDL to rat hepatocytes. Cells were prepared from control and cholestyramine-treated animals and binding studies were performed as described in Fig. 4 using identical preparations of rat ^{125}I-labeled apoE-free LDL and rat ^{125}I-labeled apoE-rich HDL. Total binding represents binding of 10 μg ^{125}I-labeled lipoprotein alone, while specific "competitive" binding was binding displaced by a 100-fold excess of unlabeled competitor. (A) total rat ^{125}I-labeled apoE-free LDL binding; (B) specific rat ^{125}I-labeled apoE-free LDL binding; (C) total rat ^{125}I-labeled apoE-rich HDL binding; and (D) specific rat ^{125}I-labeled apoE-rich HDL binding. Each value represents the mean ± SD from n = 4 cell dishes from the same preparation of hepatocytes.

mediated uptake is necessary. Furthermore, characterization of the binding of rat serum lipoproteins indicates that specific ("competitive") binding sites recognize both apoB and apoE, suggesting that on cultured rat hepatocytes common receptors are responsible for binding both apoB- and apoE-containing lipoproteins.

Previous studies using cultured rat hepatocytes showed that bile acid synthesis varies in parallel with changes in intracellular cholesterol concentrations (29). Several mechanisms were used to increase hepatocyte cholesterol availability: increasing cholesterol synthesis in response to mevalonic acid treatment, intake of dietary cholesterol, and internalization of serum lipoprotein cholesterol. All these manipulations led to parallel increases in bile acid synthesis. In marked contrast, treatment of cells with mevinolin to inhibit cholesterol synthesis decreased the secretion of bile acids (29). The combined data from cultured rat hepatocytes indicate that the rate of bile acid synthesis is determined, at least in part, by the availability of cholesterol. The ability of the hepatocyte to respond to changes in cholesterol availability has led to the hypothesis that the liver can sense changes in whole body cholesterol pools via uptake of serum lipoproteins, leading to appropriate changes in bile acid synthesis (29). Hepatic lipoprotein uptake and stimulation of bile acid synthesis may provide a mechanism to maintain cholesterol homeostasis.

In this study, experiments were designed to examine whether LDL has the ability to deliver cholesterol to hepatocytes and stimulate bile acid synthesis. The data demonstrate that both human and rat LDL are capable of stimulating bile acid synthesis, increasing the accumulation of cholesteryl ester, and inhibiting the incorporation of [2-^{14}C]acetate into cholesterol (Table 1, Fig. 1). To our knowledge this is the first report showing that receptor-mediated uptake of LDL by cultured rat hepatocytes leads to a stimulation of bile acid synthesis.

The stimulation of bile acid synthesis caused by homologous apoE-free LDL was similar to that observed with apoE-rich HDL (Fig. 3B). Thus, either apoB or apoE can direct the uptake of lipoproteins to hepatocytes leading to increased bile acid synthesis. In contrast, apoA-I-rich human HDL, that was devoid of apoE, was unable to stimulate bile acid synthesis (11). This finding, together with the results showing that methylation blocks the ability of both apoE-rich HDL (11) and LDL (Fig. 3) to stimulate bile acid synthesis, suggests that in cultured rat hepatocytes receptor-mediated uptake directed by apoE or apoB is required for this stimulation. The metabolic data are corroborated by binding studies showing that apoB and apoE compete with each other to the same degree for binding to cultured rat hepatocytes. At apparent saturation, apoE and apoB were found to compete equally for a finite number of binding sites on the surface of cultured rat hepatocytes (Fig. 4). However, apoE appears to have a higher affinity for binding than does apoB. Based on total protein, apoE-rich HDL is fivefold more effective at inhibiting the binding of both ^{125}I-labeled apoE-rich HDL and ^{125}I-labeled apoE-free LDL (Fig. 4). These data are consistent with those of Innerarity and Mahley (36) who showed that the affinity of apoE HDLc is 10 to 100-fold greater than canine or human LDL in human fibroblasts.

While we cannot rule out the possibility that the lipoprotein receptors expressed by cultured rat hepatocytes have been altered during the isolation or culturing conditions, evidence is provided in support of our model as being representative of the in vivo situation. To show that the lipoprotein receptors expressed by the cultured rat hepatocyte model would faithfully express a physiologic change in receptor expression, donor rats were fed cholestyramine, a drug known to induce hepatic LDL receptors (34–36). Hepatocytes obtained from cholestyramine-fed rats displayed an increased specific ("competitive") binding for both apoE-rich HDL and apoE-free LDL (Fig. 5), consistent with an increase in the receptor(s) which recognize both particles.

The processes through which the liver clears lipoproteins from plasma remain poorly defined. However, it is known that several distinct receptors are involved. The LDL receptor can bind LDL, chylomicron remnants, apoE HDLc, and β-VLDL remnants (30–37). Perhaps the most dramatic example of the importance of the liver LDL receptor in the clearance of apoB (LDL) is the finding that transplantation of a normal liver into a receptor-negative homozygous familial hypercholesterolemic patient led to the rapid normalization of LDL clearance and plasma LDL concen-

trations (38). An alternate pathway for the selective delivery of apoE-deficient HDL cholesteryl ester to the liver exists, and is thought to involve a selective uptake of lipoprotein cholesterol at 3–4 times the rate of internalization of the apolipoprotein (39–41). To our knowledge, no data exist showing that selective uptake of cholesteryl esters affects bile acid synthesis. The liver is also responsible for the clearance of chylomicrons via a saturable process involving apoE (31, 42–44). LDL receptor-deficient patients appear to have little or no accumulation of chylomicron remnants, suggesting that a receptor in addition to the LDL receptor may be responsible for the clearance of chylomicrons (45). Nagata, Chen, and Cooper (31) showed that an antibody to the LDL receptor blocked the uptake, but not the binding of β-VLDL to rat hepatocytes. This finding was interpreted to suggest that a noninternalized receptor (i.e., glycosaminoglycan) may play a role in lipoprotein binding to liver surface membranes (31). Recently, a gene product other than the LDL receptor which shares homology to the cysteine-rich regions of the N-terminal portion of the LDL receptor has been identified (46). Whether or not this gene product functions as a lipoprotein receptor remains to be established. Lipoprotein recognition and internalization by the liver involve a complex set of receptor interactions dependent on the apolipoprotein and lipid composition of a particular lipoprotein particle. It is known that the expression of monoclonal antibody epitopes to apoB and apoE on different lipoproteins is quite variable suggesting that apolipoprotein conformation may affect receptor binding domains (47–52). Because the expression of receptor binding domains on apoE contained on chylomicron remnants may be quite different from those expressed by apoE-rich HDL, our studies do not directly address the question of whether or not an apoE-chylomicron remnant receptor exists on rat hepatocytes.

While we have demonstrated a clear ability of LDL and apoE-rich HDL to deliver cholesterol to hepatocytes and stimulate bile acid synthesis, it is important to emphasize that sources of cholesterol for bile acid synthesis can vary depending upon the rate of cholesterol biosynthesis by the liver and the amount of lipoprotein cholesterol cleared by the liver. Since LDL receptor-negative patients have normal rates of bile acid synthesis (53), it is clear that mechanisms for delivering cholesterol into the bile acid synthetic pathway other than LDL receptor-mediated uptake exist. Alternative pathways are likely to include LDL receptor-independent mechanisms, in addition to changes in hepatocyte cholesterol synthesis and utilization.

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