Bile Salt Stimulated Cholesterol Esterase Increases Uptake of High Density Lipoprotein-Associated Cholesteryl Esters by HepG2 Cells†

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ABSTRACT: Bile salt stimulated cholesterol esterase is predominantly synthesized in the pancreas. However, this enzyme is also synthesized by the liver and was found to be present in plasma. The physiologic role of the systemic cholesterol esterase has not been clearly defined. In the current study, the human hepatoma cell line HepG2 was used as a model to determine the role of cholesterol esterase on hepatic uptake of high density lipoprotein (HDL)-associated cholesteryl esters. The results showed that hepatic uptake of the cholesteryl esters analog [^{3}H]cholesteryl ether on reconstituted HDL was inhibited by anti-cholesterol esterase antibodies. The HDL-associated cholesteryl ester transported to HepG2 cells was also increased 2-fold in the presence of taurocholate, an activator of the cholesterol esterase. These results suggest that liver-derived cholesterol esterase may play an important role in cellular uptake of cholesteryl esters from HDL. This hypothesis was supported by demonstrating the ability of exogenously added cholesterol esterase to further enhance hepatic uptake of HDL-associated cholesteryl esters. The results of the current study also showed that cholesterol esterase increased free-to-esterified cholesterol ratio in the lipoprotein. Thus, alteration of HDL structure and composition contributes to the cholesterol esterase-induced cellular uptake of HDL-associated cholesteryl esters. On the basis of these observations, we propose that liver-derived cholesterol esterase may play an important role in lipoprotein metabolism.

Epidemiological evidence have shown that a low density lipoprotein (LDL) level is positively correlated with the incidence of coronary heart disease, while a high level of high density lipoprotein (HDL) is negatively correlated with the disease (Kannel et al., 1976). The current hypothesis depicts that HDL inhibit atherogenesis by promoting cholesterol efflux from peripheral tissues and transporting excess cholesterol to the liver for re-utilization and excretion (Barter, 1993). Previous studies have documented that HDL may serve as an acceptor for cholesterol efflux from lipid-laden cells such as the macrophages and smooth muscle cells (Oram, 1986). Cholesterol acquisition by HDL requires the addition of apolipoprotein (apo) E for the expansion of the lipoprotein core (Koo et al., 1985). The apo-E and cholesterol-rich HDL can then be transported to the liver via receptor-dependent endocytosis mechanisms (Mahley, 1988).

A second pathway for clearance of HDL-associated cholesteryl esters is the selective uptake mechanism by which HDL-associated neutral lipids are internalized independent of intact lipoprotein particle uptake (Pittman et al., 1987). The selective uptake of HDL cholesteryl esters is also operational in a variety of cell types in various species (Glass et al., 1983a, 1985). Although *in vitro* studies have shown that the selective uptake mechanism is a saturable process independent of endocytosis (Pittman et al., 1987), the precise mechanism for cellular uptake of the cholesteryl esters in HDL remains unknown. Previous studies indicated that lipolytic enzymes, such as hepatic lipase and phospholipase, enhanced the selective uptake of cholesteryl esters on HDL by liver cells *in vitro* (Bamberger et al., 1983, 1985; Collet et al., 1988; Kadowaki et al., 1992; Marques-Vidal et al., 1994). However, these lipolytic enzymes are not present in the plasma compartment. Thus, the physiological significance of these observations remains unclear.

Previous results from our laboratory as well as others showed the presence of a lipolytic enzyme in plasma with characteristics similar to that of the bile salt stimulated cholesterol estersae made by the pancreas (Brodt-Eppley et al., 1995; Harrison, 1988). We have also shown that the liver is capable of synthesizing the same bile salt-stimulated cholesterol esterase (Camulli et al., 1989; Kissel et al., 1989). Winkler et al. (1992) further demonstrated that the majority of the hepatic bile salt-stimulated cholesterol esterase activity was secreted from liver cells. Taken together, these results suggest that the plasma cholesterol esterase may be derived from hepatic secretion and participate in lipoprotein metabolism. Since the cholesterol esterase has also been shown to be an extracellular sterol carrier protein (Myers-Payne et al., 1995) and transport cholesteryl esters through cell membranes (Huang & Hui, 1990), cholesterol esterase secreted by the liver may function similarly in catalyzing cellular uptake of neutral lipids from the extracellular environment. The purpose of this investigation is to determine the role of cholesterol esterase in selective uptake of HDL-associated cholesteryl esters by liver cells.

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EXPERIMENTAL PROCEDURES

Materials. Freshly frozen pancreas of male Sprague-Dawley rats were obtained from Hilltop Laboratory Animals (Scottdale, PA) and were stored frozen at −70 °C until use. Chromatography matrices, including Sephadex G25, Sephacryl S300, hydroxylapatite, and heparin-Sepharose, were products of Pharmacia Biotech Inc. (Piscataway, NJ). The Baculovirus transfer vector pVL1392 and the Sf9 cells were obtained from Invitrogen Corp. (San Diego, CA). Glutamine and RPMI medium were obtained from Gibco BRL (Grand Island, NY). Fetal bovine serum was purchased from HyClone (Logan, UT). The [^{125}I]- and [^{3}H]cholesteryl hexadecyl ethers were obtained from DuPont Research Products (Boston, MA), while the [^{3}H]cholesteryl oleate was obtained from Amersham Life Science Inc. (Arlington Heights, IL). Cholesterol and cholesteryl ester analysis kits were purchased from Wako Pure Chemicals (Richmond, VA). The 4−30% gradient polyacrylamide gels used for nondenaturing electrophoresis was obtained from IsoLabs (Akron, OH). All other reagents were of the highest quality obtained from either Sigma Biochemicals (St. Louis, MO) or Fisher Chemicals (Cincinnati, OH).

Lipoprotein Preparation. Human lipoproteins were obtained from the blood of normal human subjects after an overnight fast. The LDL fractions and HDL_3 fraction were isolated by ultracentrifugal flotation between 1.02 and 1.063 g/mL and 1.125 and 1.21 g/mL, respectively, as described (Havel et al., 1955). The HDL_3 fraction was further purified by recentrifugation at 59 000 rpm for 24 h in a Beckman 70Ti rotor. The purified LDL and HDL were dialyzed exhaustively against 150 mM NaCl containing 0.05% EDTA, pH 7.4. Analysis of the isolated HDL_3 by SDS−polyacrylamide gel electrophoresis (Laemmli,1970) revealed that apo-AI is the predominate apolipoprotein and the sample did not contain any detectable apo-E.

Preparation of Labeled HDL. Neutral lipids were extracted from the HDL_3 using the potato starch−heptane delipidation method described by Krieger et al. (1978). The partially delipidated HDL_3 was reconstituted with cholesteryl esters containing tracer amount of either [^{3}H]cholesteryl hexadecyl ether or [^{3}H]cholesteryl oleate using the method described by Glass et al. (1983a). The neutral lipids not incorporated into HDL were separated from the lipoproteins by centrifugation at $d = 1.063$ g/mL at 59 000 rpm for 24 h in a Beckman 70Ti rotor. The infranatant fraction containing the radiolabeled HDL was isolated and dialyzed against saline−EDTA. The apolipoproteins associated with the HDL_3 were radiolabeled with ^{125}I using iodine monochloride (Innerarity et al., 1986).

Cholesterol Esterase and Anti-Cholesterol Esterase Isolation. Bile salt-stimulated cholesterol esterase was isolated from rat pancreas or human milk using the method as described by Jacobson et al. (1990) with minor modifications (DiPersio et al., 1992). The homogeneity of purified protein was verified by SDS−polyacrylamide gels with the observation of a single band corresponding to $M_r = 74\,000$ and 130 000 for the rat and human cholesterol esterase, respectively.

For production of antibodies against the bile salt-stimulated cholesterol esterase, a 3 kg New Zealand white rabbit was injected subcutaneously with 250 μg of purified rat cholesterol esterase mixed with complete (first injection) or incomplete (subsequent injections) adjuvant at 2-week intervals. After the third injection, the rabbit was bled to collect serum, which was stored at −70 °C until use. Specificity of the antibodies against cholesterol esterase was based on reactivity with a single band with molecular weight of purified cholesterol esterase upon immunoblotting with pancreatic extracts (Huang & Hui, 1991). The anti-cholesterol esterase IgG was effective in immunoprecipitation of cholesterol esterase specifically from rat pancreatic acinar cells (Huang & Hui, 1994) and in inhibition of its cholesteryl ester hydrolytic activity (data not shown).

Mutagenized Cholesterol Esterase. Cholesterol esterase containing the H435Q ($His^{435}\rightarrow Gln^{435}$) mutation was produced by recombinant Baculovirus transfected Sf9 cells as described (DiPersio et al., 1992). Briefly, a mutagenized cholesterol esterase cDNA with a glutamine-to-histidine substitution at residue 435 was subcloned into the baculovirus transfer vector pVL1392 and used to transfect Sf9 cells in the presence of *Autographa californica* nuclear polyhedrosis virus DNA. Isolation of the recombinant baculovirus and the subsequent expression and purification of recombinant cholesterol esterase were performed as described (DiPersio et al., 1992). The normal and the mutagenized form of cholesterol esterase displayed similar electrophoretic mobility on SDS−polyacrylamide gels. The recombinant protein was dialyzed in buffer A and stored at −70 °C until use.

Cell Culture. Stock cultures of the human hepatoma cell line HepG2 were grown in RPMI 1640 medium supplemented with 2 mM glutamine and 10% fetal bovine serum. The cells were maintained in 75 cm^2 flasks at 37 °C in a humidified incubator at 5% CO_2. When the cell culture reached 80% confluence, the cells were dissociated with 0.25% trypsin and 0.05% EDTA and replated into new flasks at a split ratio of 1:4. The cells received fresh medium every 2 days. For experimentation, the cells were plated on six-well tissue culture plates at a density of approximately $2-3 \times 10^4$ cells/cm^2. Experiments were performed 5−6 days after plating when the cells reached confluence.

On the day of experiment, fresh medium containing the radiolabeled HDL was added to the cells, and incubation was carried out for 4 h at 37 °C as described in legend of appropriate figure. At termination of the experiment, the medium was removed, and the cells were washed and chased with medium exactly as described (Oram, 1986). The cells were finally dissolved in 1 M NaOH and assayed for radioactivity.

Modification of HDL by Cholesterol Esterase. HDL (500 μg of protein) was incubated with or without cholesterol esterase (50 μg of protein) or the H435Q mutagenized cholesterol esterase for 4 h at 37 °C in the presence or absence of 2 mM taurocholate. The modified HDL was separated from the cholesterol esterase by centrifugation at $d = 1.21$ g/mL at 40 000 rpm for 12 h in a Beckman 50.3Ti rotor. Free cholesterol and total cholesterol of the reisolated HDL were determined by enzymatic colorimetric method using an enzymatic kit obtained from Wako.

RESULTS

In agreement with results reported by other investigators (Winkler et al., 1992), conditioned medium from HepG2 cells displayed significant bile salt-dependent cholesteryl ester hydrolytic activity (Figure 1). In view of previous studies

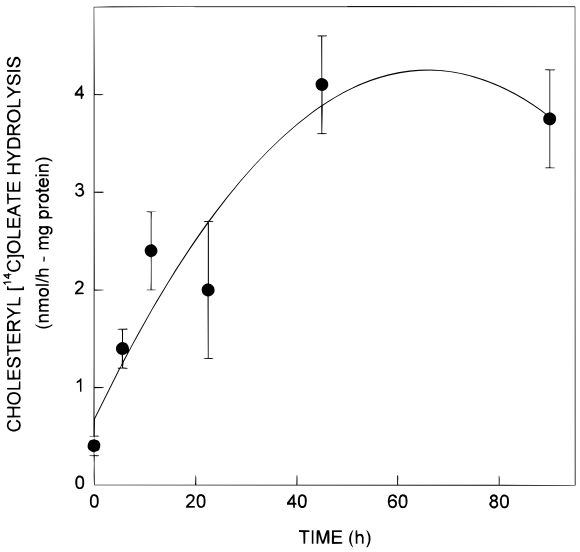

FIGURE 1: Cholesteryl ester hydrolytic activity secreted by HepG2 cells. The HepG2 cells were incubated in RPMI 1640 medium at 37 °C, and an aliquot of the medium was collected at the indicated time. Cholesteryl ester hydrolytic activity was determined by incubating an aliquot of the medium for 4 h at 37 °C with cholesterol [^{14}C]oleate in a 200 μL reaction containing 50 mM Tris-HCl (pH 7.5) and 2 mM taurocholate. The reaction was terminated by adding 1.05 mL of 50 mM sodium carbonate and 50 mM sodium borate (pH 10). The reaction product, [^{14}C]oleate was extracted as the free fatty acid with 3.25 mL of methanol/chloroform/heptane (1.41:1.25:1.0). The data represent results from triplicate reaction assays ± SEM.

demonstrating pancreatic cholesterol esterase mRNA in liver RNA preparations (Kissel et al., 1989; Zolfaghari et al., 1989), additional experiments were warranted to determine if the enzyme activity secreted by HepG2 cells was due to secretion of this protein. In preparation for the experiments, antibodies were prepared against purified pancreatic cholesterol esterase for immunoblotting assays. The specificity of the antibody for the pancreatic cholesterol esterase was confirmed based on its reactivity with one major protein, with electrophoretic mobility similar to that of purified cholesterol esterase (Figure 2). The immunoreactivity of the minor bands at lower molecular weight was found to increase upon storage of the sample, suggesting that these proteins were probably degradative products of the cholesterol esterase.

The immunoblot of HepG2 conditioned medium with the anti-cholesterol esterase revealed a single immunoreactive protein (Figure 2). The molecular weight of this immunoreactive protein, determined to be ~130 000, was similar to that of purified human cholesterol esterase (Figure 2). These results suggested that the cholesterol esterase secreted by the HepG2 cells was similar and probably identical to the pancreatic enzyme and the enzyme secreted into human milk. The presence of the pancreatic type cholesterol esterase in liver was also demonstrated using conditioned medium from the rat hepatoma McA-RH7777 (data not shown). Although the level of cholesterol esterase secreted by the rat hepatoma cells was much lower than that observed for the human HepG2 hepatoma cell line, the synthesis of the pancreatic cholesterol esterase by two independent hepatoma cell lines suggested that this protein is a normal secretory product of liver cells.

The potential role of the secreted cholesterol esterase in catalyzing cellular uptake of lipoprotein-associated lipids was

FIGURE 2: Immunoblot of cholesterol esterase secreted by HepG2 cells. The HepG2 cells were incubated in RPMI 1640 medium for 48 h at 37 °C. At the end of the incubation period, the medium was collected, dialyzed against distilled water, and lyophilized. The sample was dissolved in 200 μL of water and then applied to SDS−polyacrylamide gels. The proteins were transferred to nitrocellulose paper and incubated with anti-cholesterol esterase antibodies at a dilution of 1:1000. Immunoreactive bands were visualized by incubating the blots with an ^{125}I-labeled antibodies against rabbit IgG. Lanes A and B represent 1 and 5 μg of purified human cholesterol esterase, respectively. Lanes C and D are 50 and 150 μL samples of HepG2 medium.

explored by studying the influence of anti-cholesterol esterase IgG on the selective uptake of HDL-associated cholesteryl esters. In these experiments, the HepG2 cells were incubated for 4 h at 37 °C with HDL (50 μg/mL of HDL protein) containing either [^{3}H]cholesteryl hexadecyl ether or ^{125}I-labeled apolipoproteins. At the end of the incubation period, the cell-associated radioactive tracer was determined as apparent uptake of the entire lipoprotein particle, reported based on lipoprotein protein, for direct comparison of the rates of uptake of the various constituents in the same lipoprotein as described (Rinninger & Pittman, 1988). The results showed that HepG2 cells were capable of internalizing an apparent 490 ng of HDL protein per mg of cell protein, if [^{3}H]cholesteryl hexadecyl ether was used as the tracer label. The apparent HDL uptake was only 19.2 ng per mg of cell protein if ^{125}I-labeled HDL protein was used as the tracer label (Figure 3). These results confirmed previous reports of selective uptake of HDL-associated cholesteryl esters by HepG2 cells (Rinninger & Pittman, 1988). Interestingly, addition of 2 mM taurocholate, an activator of cholesterol esterase, resulted in substantial increase in the amount of [^{3}H]cholesteryl ether taken up by the cells, while the addition of anti-cholesterol esterase antibodies to the incubation medium, in the presence or absence of added taurocholate, dramatically decreased cellular uptake of the radiolabeled lipids, but not the apolipoproteins, from HDL (Figure 3). A maximum of 55% inhibition of selective HDL

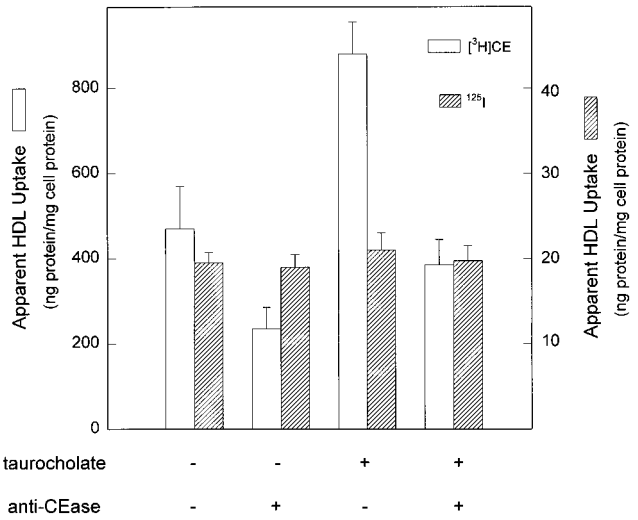

FIGURE 3: Effects of taurocholate and anti-cholesterol esterase on selective uptake of HDL-associated cholesteryl esters. The HepG2 cells were incubated for 4 h at 37 °C with HDL (35 μg/mL of protein) containing either [^{3}H]cholesteryl ether or ^{125}I-labeled proteins in the presence or absence of 2 mM taurocholate or 1:1000 dilution of anti-cholesterol esterase antiserum. The amount of ^{3}H-labeled lipids (open bars) or ^{125}I-labeled proteins (striped bars) taken up by the cells was determined and expressed as apparent HDL uptake if the intact HDL was internalized by the cells. Results presented were average of triplicate determinations from three different experiments ± SEM.

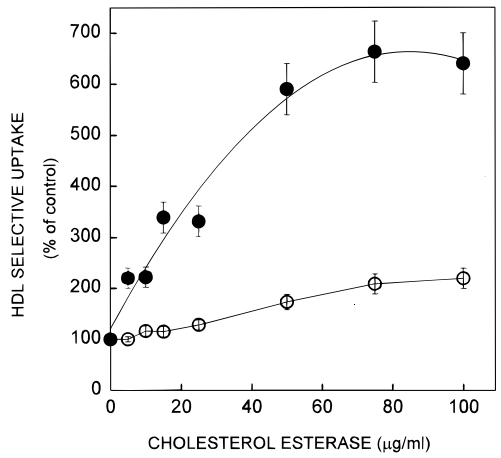

FIGURE 4: Concentration dependence of cholesterol esterase-induced selective uptake of HDL-associated lipids by HepG2. The HepG2 cells were incubated for 4 h at 37 °C with [^{3}H]cholesteryl ether-labeled HDL (35 μg/mL of protein) with cholesterol esterase in the presence (●) or absence (○) of 2 mM taurocholate. Stimulation of selective uptake of HDL-associated radiolabeled lipids was reported as percent of uptake in the absence of exogenously added cholesterol esterase. The 100% control level averaged 490 ng of apparent HDL uptake per mg of cell protein. The results were averaged of triplicate determinations from two different experiments ± SEM.

cholesteryl ester uptake was observed with 1:1000 dilution of the antiserum. Increasing concentrations of the antibody did not result in further inhibition of the selective uptake process. These results suggested that cholesterol esterase secreted by the HepG2 cells may participate in lipid transport by facilitating the selective cellular uptake of HDL-associated cholesteryl esters. However, it must be noted that the selective uptake process did not require the presence of the exogenous cholesterol esterase.

Consideration was given to the possibility that the taurocholate augmented selective uptake was due to detergent effects of the bile salt on lipoproteins and/or the hepatoma cells. Therefore, two different experiments were performed to examine this possibility. In the first experiment, inclusion of 2 mM octyl glucoside, a nonionic detergent with similar critical micellar concentration as taurocholate, did not show any effect on the selective uptake of HDL-associated cholesteryl esters by HepG2 cells. The second experiment showed that stimulation by taurocholate on the selective uptake process could be inhibited totally by addition of anti-cholesterol esterase to the incubation medium (Figure 3). Therefore, the bile salt induced selective uptake was likely due to activation of cholesterol esterase secreted by HepG2 and was not due to nonspecific effects.

The cholesterol esterase-stimulated selective uptake of HDL-associated cholesteryl esters was dependent on enzyme concentration in the medium. When the HepG2 cells were incubated with 35 μg/mL of [^{3}H]cholesteryl ether-labeled HDL in the presence of 0−100 μg/mL of purified cholesterol esterase, a small but significant induction of the selective uptake process was observed. A consistent 2-fold induction of selective uptake was observed with 50−100 μg/mL of cholesterol esterase in the absence of bile salt (Figure 4). The activation of cholesterol esterase by submicellar concentrations of bile salt, such as 2 mM taurocholate, resulted in further stimulation of selective uptake, reaching a maximum level of 6−7-fold induction at 50−100 μg/mL of cholesterol esterase (Figure 4).

Taurocholate-dependent cholesterol esterase stimulation of selective uptake suggested that enzymatic activity was required for maximum stimulation. This hypothesis was examined further by comparing the ability of native enzyme with that of an inactive mutagenized cholesterol esterase to stimulate selective uptake of HDL lipids. In this experiment, the HepG2 cells were incubated with [^{3}H]cholesteryl ether-labeled HDL in the presence of 25 μg/mL of recombinant cholesterol esterase, containing either the native sequence or with a His^{435}→Gln^{435} substitution (DiPersio et al., 1991). The amount of radiolabeled lipids taken up by the HepG2 cells were determined after 4 h of incubation as described in Experimental Procedures. The results showed that recombinant cholesterol esterase containing the native sequence was similar to rat cholesterol esterase in its ability to stimulate HepG2 uptake of HDL-associated neutral lipids (Figure 5). In contrast, the His^{435}→Gln^{435} mutant, which was shown to be enzymatically inactive (DiPersio et al., 1991), was ineffective in stimulating the selective uptake of radiolabeled cholesteryl ether from HDL (Figure 5).

The possible role of HDL-associated cholesteryl ester hydrolysis HDL by the cholesterol esterase in the selective uptake process was investigated. In this experiment, the HepG2 cells were incubated for 4 h at 37 °C with HDL containing either [^{3}H]cholesteryl hexadecyl ether or [^{3}H]-cholesteryl oleate. At the end of incubation, the cell-associated radioactivity was determined to reflect total amount of HDL protein taken up by the cells. The results showed that the apparent uptake of HDL was 2-fold higher if [^{3}H]cholesteryl oleate instead of radiolabeled cholesterol ether was used as the tracer (Figure 6). Thus, cholesterol esterase was more effective in inducing selective uptake of cholesteryl oleate than that of cholesteryl ether (Figure 6). Nevertheless, as reported above, cholesterol esterase was also effective, albeit to a lesser extent, in facilitating selective uptake of the nonhydrolyzable cholesteryl ether from HDL.

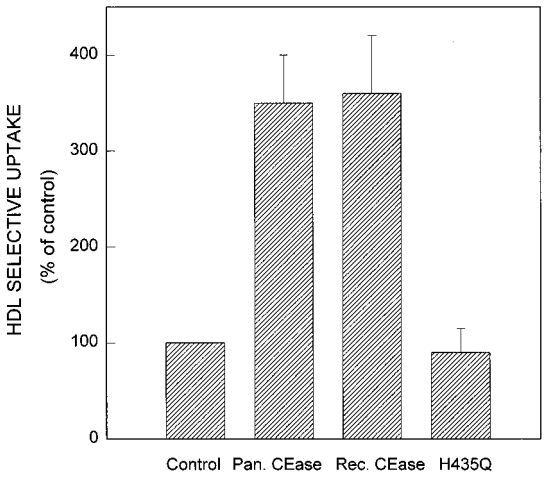

FIGURE 5: Effects of various cholesterol esterase preparations on selective uptake of HSL-associated lipids by HepG2 cells. The HepG2 cells were incubated for 4 h at 37 °C with [^{3}H]cholesteryl ether-labeled HDL (35 μg/mL of protein) and 2 mM taurocholate in the absence of exogenously added cholesterol esterase (control) or in the presence of 25 μg/mL of either purified rat pancreatic cholesterol esterase (pan. CEase), recombinant form of rat cholesterol esterase (Rec. CEase), or a mutagenized rat cholesterol esterase (H435Q). Stimulation of selective uptake of HDL-associated radiolabeled lipids was reported as percent of uptake in the absence of exogenously added cholesterol esterase. The 100% control level averaged 490 ng of apparent HDL uptake per mg of cell protein. The results were averaged of triplicate determinations from two different experiments ± SEM.

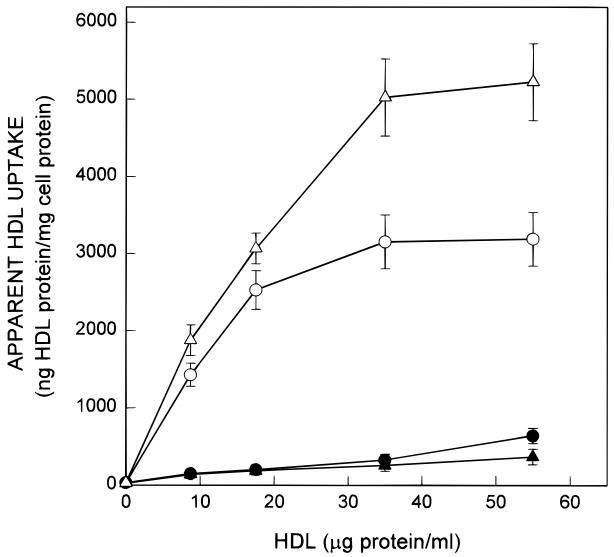

FIGURE 6: Effects of cholesterol esterase-induced hydrolysis of cholesteryl esters on selective uptake of neutral lipids by HepG2 cells. The HepG2 cells were incubated for 4 h at 37 °C with HDL containing either [^{3}H]cholesteryl ether (●, ○) or [^{3}H]cholesteryl oleate (▲, △) in the presence (○, △) or absence (●, ▲) of 2 mM taurocholate. The amount of ^{3}H-labeled lipids taken up by the cells was determined and expressed as apparent HDL uptake if the intact HDL was internalized by the cells. Results presented were average of triplicate determinations from two different experiments ± SEM.

Taken together, these results suggest that hydrolysis of the cholesteryl esters in HDL may be one step by which cholesterol esterase increased selective uptake of cholesterol and cholesteryl esters from HDL.

The observations for the requirement of cholesteryl ester hydrolytic activity and the ability of cholesterol esterase to increase the selective uptake of the nonhydrolyzable cholesterol ether appeared to be a direct conflict. However, it is possible that the cholesterol esterase-induced cellular uptake of cholesterol ether was due to its ability to hydrolyze cholesteryl esters and alter the structure and composition of the lipoprotein. To test this hypothesis, HDL was incubated for 4 h at 37 °C in the presence or absence of 2 mM taurocholate, with or without cholesterol esterase or with the H435Q mutagenized cholesterol esterase. The incubation was terminated by raising the density of the solution to 1.21 g/mL and then centrifuged at 40 000 rpm for 12 h in a Beckman 50.3Ti rotor. Composition and structure of the reisolated HDL were determined as described in Experimental Procedures. The results showed that total cholesterol recovery was similar (approximately 60% in each case) when HDL was incubated with or without cholesterol esterase or with the mutagenized inactive enzyme. However, the amount of free cholesterol in HDL increased 10-fold while the amount of total cholesteryl esters decreased to 33% when the lipoprotein was incubated with native cholesterol esterase. These results confirmed the effects of cholesterol esters on HDL structure and composition, which may dictate the efficiency of its uptake by hepatic cells.

DISCUSSION

The selective uptake of HDL cholesterol was demonstrated in a variety of tissues including the liver. In a series of elegant studies by Pittman and his associates (Glass et al., 1983b, 1985; Pittman & Steinberg, 1984; Pittman et al., 1987; Rinninger & Pittman, 1987; Knecht & Pittman, 1989), the transport of HDL cholesterol to the cells was shown to be independent from cellular uptake of the HDL apolipoproteins. These investigators also showed that the selective uptake of HDL-associated cholesteryl esters was not inhibited by chloroquine, monensin, and colchicine, suggesting that endocytosis was not required for this process (Pittman et al., 1987). Although the precise mechanism for the selective uptake of HDL-cholesteryl esters remains unknown, it appears to be a two-step process involving a reversible and an irreversible pool of neutral lipids (Knecht & Pittman, 1989; Rinninger et al., 1993). Knecht and Pittman (1989) postulated that the cholesteryl ester in the reversible pool is associated with plasma membranes and may serve as the precursor for cholesteryl ester in the irreversible pool. More recently, Acton et al. (1996) showed that the initial process for the selective uptake of HDL cholesteryl ester involves binding of the lipoproteins to the SR-B1 receptor. Data presented by other laboratories indicated that the cholesteryl ester taken up through the selective uptake pathway is hydrolyzed outside the lysosomal compartment by a neutral cholesteryl ester hydrolase (Sparrow & Pittman, 1990; DeLamatre et al., 1991, 1993). Thus, a key step in hepatic uptake of HDL cholesteryl esters may be their hydrolysis in the reversible pool prior to transport to an internal irreversible pool. The inhibition of cholesteryl ester hydrolysis with diethylumbelliferyl phosphate has been shown to result in significant reduction of HDL cholesteryl ester uptake by a rat hepatoma cell line (DeLamatre et al., 1993). Thus, the hydrolysis of the cholesteryl ester in HDL may enhance its interaction with the SR-B1 HDL receptor on the cell surface and facilitate the selective uptake process.

Results reported in this communication documented that bile salt-stimulated cholesterol esterase synthesized by the liver participates in the hepatic uptake of HDL-associated

cholesteryl esters. The ability of endogenous cholesterol esterase to mediate the selective transport process was demonstrated by the ability of the anti-cholesterol esterase antibodies to inhibit the selective uptake process. The selective uptake of HDL-associated cholesteryl esters could be enhanced by addition of exogenous cholesterol esterase to the incubation medium, suggesting that the level of cholesterol esterase may be important in regulating the amount of cholesteryl esters transported to the cells. Additionally, results of this study also showed that the selective cholesteryl ester transport in hepatoma could be increased by taurocholate, a bile salt which enhanced the activity of the cholesterol esterase. Although taurocholate can serve as a detergent and facilitates cholesteryl ester transport by solubilization of the neutral lipids in the lipoproteins, the optimum concentration of taurocholate for stimulation of cholesteryl ester uptake was 2 mM. This concentration was substantially lower than the critical micellar concentration of taurocholate. Therefore, the detergent effect at 2 mM taurocholate was minimal. On the other hand, this concentration of taurocholate was shown previously to be optimal in stimulation of hepatic cholesterol esterase activity (Camulli et al., 1989). More importantly, the stimulation of selective uptake by taurocholate was inhibited by antibodies against cholesterol esterase. Taken together, these observations suggested that taurocholate stimulates the selective uptake of HDL-associated cholesteryl esters by activation of the hepatic cholesterol esterase.

The requirement of bile acids for the optimal transport of HDL cholesteryl esters to the liver suggests that cholesterol esterase hydrolytic activity, involving alteration in HDL structure and composition, is required for this process. This hypothesis is consistent with the observations that an inactive mutagenized cholesterol esterase failed to facilitate HDL lipid transport to the liver. However, our data also showed that, while hydrolysis of the substrate is required for optimal stimulation of the selective uptake process, enzymatic activity is not essential for the enzyme delivery of cholesteryl ester substrates to the cells. The latter conclusion is supported by evidence showing the ability of cholesterol esterase to facilitate cellular uptake of cholesteryl ether, a nonhydrolyzable analog of esterified cholesterol. These results suggest that the cholesterol esterase may facilitate the initial step of the selective uptake pathway by serving as a carrier protein in delivering cholesteryl esters to the cell membrane (Myers-Payne et al., 1995). Further hydrolysis of the cholesteryl esters may be necessary for maximal transport, possibly through delivery of the lipids to the irreversible pool. The inhibition by polyclonal anti-cholesterol esterase suggests that the antibody preparation contains IgG with specific epitopes against both the carrier activity and the hydrolytic activity of the cholesterol esterase.

The experimental data presented herein also showed that cholesterol esterase only facilitates HDL cholesterol transport to the liver and that the selective uptake process occurs in the absence of the protein and/or its activity. Thus, although cholesterol esterase is present in the serum, the low concentration of bile salt in peripheral circulation is likely to preclude the involvement of cholesterol esterase in the selective uptake of HDL lipids by peripheral tissues. However, bile acid concentrations in the portal blood were reported to be in the millimolar range (Murphy, 1988), which is sufficient for cholesterol esterase activation. Thus, in the sinusoids of the liver where cholesterol esterase can be mixed with bile acids, the enzyme can play a major role in facilitating the selective hepatic uptake of cholesterol and cholesteryl esters from HDL. The hypothesis is consistent with previous studies showing an increased hepatic uptake of cholesterol and cholesteryl esters after bile acid infusion into the jugular vein (Bravo & Cantafora, 1990). Taken together, these results indicate that cholesterol esterase synthesized in the liver and present in plasma may participate in lipoprotein metabolism by facilitating the reverse cholesterol transport process in delivering extrahepatic cholesterol from HDL to the liver.

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