Endocytosis of Hepatic Lipase and Lipoprotein Lipase into Rat Liver Hepatocytes in Vivo Is Mediated by the Low Density Lipoprotein Receptor-related Protein*

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Marcel Verges‡§, Andre Bensadoun¶, Joachim Herz‡, John D. Belcher**,** and Richard J. Havel† ‡‡

From the ‡Cardiovascular Research Institute, University of California, San Francisco, California 94143, the ¶Department of Anatomy and Biochemistry and Biophysics, the §Division of Nutritional Sciences, Cornell University, Ithaca, New York 14853, and the ¶Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, Texas 75390

In isolated cells, the internalization and degradation of hepatic lipase (HL) has been linked to its binding to the low density lipoprotein receptor-related protein (LRP). We have utilized the receptor-associated protein (RAP), a universal inhibitor of high affinity ligand binding to LRP, to evaluate the participation of LRP in the endocytosis of HL and lipoprotein lipase (LPL). We isolated a total endosome fraction from rat livers after a 30-min infusion of recombinant RAP, administered as a glutathione S-transferase conjugate (GST-RAP). GST-RAP infusion had no effect on the concentration of HL in liver homogenates, but its concentration in blood plasma increased progressively by 20%, and enrichment over homogenate of HL in endosomes was reduced by 50% as compared with infusion of GST alone. The concentrations of LPL in liver and plasma were 1.4 and 0.5%, respectively, those of HL, but endosomal enrichment of the two enzymes was similar (~10-fold). GST-RAP infusion had no effect on the concentration of LPL in liver but increased its concentration in blood plasma by 250% and reduced its endosomal enrichment by 95% or greater. GST-RAP infusion also reduced endosomal enrichment of LRP by 40%, but enrichment of several other endocytic receptors was unaffected. Endosomal enrichment of several membrane trafficking proteins associated with the endocytic pathway in hepatocytes was unaffected by GST-RAP with the exception of early endosome endosome antigen 1, which was reduced by 85%. We conclude that HL is partially and LPL almost exclusively taken up into rat hepatocytes after binding to the endocytic receptor LRP.

Hepatic lipase (HL) is synthesized by hepatocytes and resides on liver cell surfaces, presumably bound to heparan sulfate proteoglycans (HSPGs) (1). HL does not hydrolyze lipids of nascent chylomicon (2), but it participates in the clearance of chylomicron remnants from the blood and hydrolysis of component triglycerides and phospholipids (3, 4). It also contributes to the conversion of hepatogenous triglyceride-rich lipoproteins to LDL and to remodeling HDL (4, 5). Chylomicron remnants are efficiently removed from the blood by binding to macromolecules on the basolateral microvilli of hepatocytes, including HL (6, 7). Binding of chylomicron remnants to HL causes a delay in the endocytosis of these particles, which is subsequently mediated by the LDL receptor and LDL receptor-related protein-1 (LRP) (6, 8). It has been postulated that remodeling of chylomicron remnant lipids by HL facilitates receptor binding via apoE (9), leading to efficient endocytosis via coated pits. In the absence of apoE, HL may provide a less efficient pathway for removal of remnant lipoproteins containing apoB-48 (10).

Rat HL binds LRP with high affinity (11). In human hepatoma cells, fibroblasts, and Chinese hamster ovary cells, internalization and degradation of HL bound to HSPGs are mediated mainly by LRP (11, 12). Lipoprotein lipase (LPL) also binds to HSPGs (13), and its internalization and degradation by human fibroblasts are dependent upon LRP (14). Initial binding of LPL to HSPGs is necessary for degradation (14, 15). In light of these observations in cultured cells, we have pursued our earlier observation (16) that HL is concentrated in hepatocytic endosomes from rat liver, and we report here evidence that LRP contributes in vivo to the endocytosis of HL destined for lysosomal degradation. We also report evidence that lipoprotein lipase (LPL), which is removed from the blood mainly by the rat liver (17), is taken up into rat hepatocytes exclusively by this endocytic receptor.

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—α2-Macroglobulin (α2MG) (Sigma) was activated with methyamine and radiiodinated (125I) as described (18). Rat 39-kDa receptor-associated protein (RAP) was encoded as a bacterial glutathione S-transferase (GST) fusion protein in the plasmid pGEX-DGRAP and prepared as described (19). Similarly encoded GST alone was used as a control.

Rabbit polyclonal anti-LRP was raised against the C-terminal cytoplasmic tail of the human protein (20). Mouse monoclonal anti-LDL receptor (IgG 4A4) was raised against the C-terminal cytoplasmic tail of the human receptor (21). Goat polyclonal anti-asialoglycoprotein receptor was raised against the rat protein (22). The following antisera against human proteins were generously provided: polymeric immunoglobulin-A (IgA, 9000); human IgM, human IgG, and mouse IgG, respectively; rabbit IgA, rabbit IgG, and mouse IgG, respectively.

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globulin receptor SC166 (I.-P. Kraushenbel, ISREC, Lausanne, Switzerland); transferring receptor H68.4 (I. Towbridge, Salk Institute, San Diego); cellubrevin (R. Jahn, Yale University, New Haven, CT); endo-brevin (W. Hong, Institute of Molecular and Cellular Biology, Singapore); and Rab11 121 (R. Parton, University of Queensland, Australia). Other antibodies were purchased commercially as follows: epidermal growth factor receptor 1005, sc-03 (Santa Cruz Biotechnology, Santa Cruz, CA); Rab5 44-332 (QBC Inc., Hopkinton, MA); early endosome antigen 1 (EEA1) E41120 and Golgi matrix protein of 130-kDa (GM130) clone 35, G56120-050 (Transduction Laboratories, Lexington, KY). Secondary antibodies were from Jackson ImmunoResearch (West Grove, PA).

Isolation of Endosomes—Initial experiments to evaluate the presence and distribution of HL in endosomes were carried out in male Sprague-Dawley rats treated with 17-α-ethinyl estradiol, which highly induces LDL receptors in hepatocytes. In these rats, three highly purified endosome fractions, early endosomes, late endosomes (multivesicular bodies), and a receptor-recycling compartment (RRC), can readily be isolated (23). The volume and mass of the multivesicular body fraction characteristically increases 15 min after injection of human LDL. Male Sprague-Dawley rats, weighing 300–350 g and treated with estradiol for 5 days as described (23), were fasted overnight and then anesthetized with isoflurane (Schering-Plough Animal Health Corp.). Before or 15 min after injection of LDL (1.0–1.5 mg of protein) through an exposed femoral vein, livers were exposed with an abdominal incision. The liver was removed, weighed, and three endosome fractions were prepared exactly as described (23). The purity was assessed by negative staining electron microscopy (24).

To determine the effect of administering RAP upon the endocytosis of HL and LPL, we used untreated male Sprague-Dawley rats weighing 230–270 g, from which we isolated a total endosome fraction from liver homogenates. Rats fasted overnight were anesthetized with a mixture of ketamine (75–95 mg/kg) and xylazine (5 mg/kg), administered intraperitoneally in a volume of 0.3 ml 0.15 M NaCl. Additional anesthetic was used as needed to maintain light anesthesia. A midline incision was made over the trachea, and a carotid artery was exposed, cannulated with PE-60 polyethylene tubing advanced 1 cm caudad through a small incision, and tied in place. The incision was closed with surgical clips. GST-RAP or GST was injected into the carotid artery as a pulse of 1 ml followed by a constant infusion with a pump at a rate of 4 ml/h. The least amount of GST-RAP required to prevent the hepatic uptake of radiiodinated α-MG over a period of 30 min, determined as described below, was utilized. Control animals received an equimolar amount of GST. Blood samples of 50–100 μl were obtained from the orbital plexus 1 and 15 min after starting the infusions and placed in tubes containing EDTA (final concentration 0.2%). At 27 min, the liver was excised, and the portal vein was cannulated with PE-50 polyethylene tubing and tied in place. At 30 min, a final blood sample was obtained from the inferior vena cava, following which the vena cava was opened and the liver flushed as described above. The chest was then opened to ensure cessation of breathing. Livers were removed, weighed, and a small portion was taken for assay of 125I.

| Enzymes | Early endosomes | Multivesicular bodies | Receptor-recycling compartment |
|---------|-----------------|-----------------------|-------------------------------|
| HL      | 13.5 ± 5.7      | 30.1 ± 8.4            | 4.1 ± 2.7                    |
| LPL     | 12.2            | 19.7                  | 2.6                          |

a Fold enrichment over liver homogenate (n = 3; mean and S.E.); homogenate activity = 5.29 ± 0.62 nmol min−1 mg protein−1.

b Fold enrichment over homogenate (n = 1); homogenate mass = 28 ng/mg protein.

and weighed, and a small portion was taken for assay of 125I. Enzymes—Protein was quantified (25) in liver fractions (homogenate and endosomes). Total cholesterol (26) and triglycerides (27) were quantified in plasma (mg/dl) and liver fractions (mg/mg protein). Rat HL (28) and LPL (29) mass were estimated by specific ELISAs in plasma (ng/ml) and liver fractions (ng/mg protein). In estradiol-treated rats, HL activity against an emulsified triglyceride substrate was also assayed in homogenate and endosome fractions (30). Values for these enzymes in endosomes are expressed as fold enrichment over homogenate. Enzyme masses in total plasma were calculated for a plasma volume of 4.5% of body weight and compared with corresponding values for total liver (ng/mg protein × total liver protein).

Proteins in liver fractions were separated by SDS-PAGE and subjected to Western blotting as described (31). Components were visualized by enhanced chemiluminescence (ECL, PerkinElmer Life Sciences). Band intensities were quantified with the IPlab Gel program (Signal Analytics Corp., Vienna, VA) and expressed as volume units/mg of protein. All estimates of enrichment in endosomes/homogenate were from the same exposure of a given gel.

**RESULTS**

**HL Is Enriched in a Characteristic Pattern in Hepatocytic Endosomal Fractions**—In rat liver, ligands taken up from the blood by high affinity receptors rapidly enter endosomal compartments in hepatocytes (32). Little HL circulates in the blood of rats, and most is present in the extracellular space of the liver (space of Disse), presumably bound to proteoglycans (3, 33). We found, however, that this protein, evaluated by its activity against triglyceride substrate and mass (estimated immunohchemically), was enriched in three endosome fractions from estradiol-treated rats in a distinctive pattern: highest in multivesicular bodies (late endosomes), intermediate in early endosomes, and lowest in receptor-recycling endosomes (Table 1). This pattern is characteristic of ligands and receptors destined for lysosomal degradation in hepatocytes, and the opposite of that was observed for receptors and ligands, such as the LDL receptor and transferrin, that recycle to the cell surface (23, 34, 35). Total HL activity recovered in multivesicular bodies increased from 0.31 ± 0.06 to 0.77 ± 0.27% of total liver homogenate (mean and S.D., n = 5) 15 min after loading the liver with human LDL, with no change in enrichment. Endosomal HL actively hydrolyzed triglycerides in endocytosed chylomicron remnants, and its activity in homogenate and endosomes was completely inhibited by goat antiserum against dog HL (data not shown). The extent of enrichment of HL in multivesicular bodies over liver homogenate (20–30-fold) was lower than that observed for ligands such as LDL (–200-fold). This presumably reflects the relatively large pool of HL on liver cell surfaces (3, 33). These results are consistent with the hypothesis that HL may leave this pool by binding to receptors that enter hepatocytes via coated pits.

**Selection of Dose of GST-RAP to Inhibit Binding of Lipoproteins to LRP**—RAP prevents binding of all high affinity ligands, such as HL and LPL, to LRP. GST-RAP has manyfold higher affinity for LRP than for the LDL receptor (18, 36). Previous studies

**TABLE I**

| Endosome fraction | Activitya | Massb |
|-------------------|-----------|-------|
| Early endosomes   | 13.5 ± 5.7| 12.2  |
| Multivesicular bodies | 30.1 ± 8.4 | 19.7  |
| Receptor-recycling compartment | 4.1 ± 2.7 | 2.6   |

a Fold enrichment over liver homogenate (n = 3; mean and S.E.); homogenate activity = 5.29 ± 0.62 nmol min−1 mg protein−1.

b Fold enrichment over homogenate (n = 1); homogenate mass = 28 ng/mg protein.
plasma.

GST-RAP in 0.15 M NaCl, 1 mg/ml (squares), or 2 mg/ml (triangles) was injected as an initial pulse of 1 ml through a carotid artery of individual anesthetized rats. GST, 0.8 mg/ml (diamonds), was injected as a control. Immediately thereafter, the rats received 50 μg of 125I-α2-MG in 0.5 ml of 0.15 M NaCl via a femoral vein, followed by a constant infusion of the corresponding GST-RAP or GST solution via the carotid artery for 30 min at a rate of 4 ml/h. Blood samples of ~50 μl were obtained from the orbital plexus at intervals after the injection of α2-MG for assay of 125I. Results are expressed as % counts/min remaining in plasma after the 1-min sample. A total of 6 mg of GST-RAP (2-mg pulse and 4 mg over 30 min) was required to block clearance of α2-MG.

(18) showed that GST-RAP is rapidly endocytosed into rat hepatocytes after intravenous injection. Accordingly, to avoid appreciable binding of GST-RAP to the LDL receptor, we first determined the smallest dose of GST-RAP, given as a pulse intravenously and followed by a continuous infusion, that would completely prevent binding of a known high affinity ligand for LRP in fasted male Sprague-Dawley rats. As shown in Fig. 1, a pulse of 2 mg of GST-RAP, followed by infusion at a rate of 0.13 mg/min for 30 min, almost completely eliminated the clearance of 50 μg of activated α2-MG, whereas a 50% lower amount of GST-RAP yielded only partial inhibition of clearance as compared with infusion of GST. The effective dose was utilized in all subsequent experiments.

Effect of GST-RAP on the Concentration of HL and LPL in Plasma and Liver and Their Enrichment in Hepatocytic Endosomes—During the 30-min infusion of GST-RAP, the concentration of HL increased progressively in blood plasma (Fig. 2A and Table II). After 30 min, the concentration of HL in plasma had increased significantly by 20% in animals given GST-RAP, whereas no change occurred in control animals given GST. However, the liver still contained 99% of the total mass of HL in liver + plasma in rats given GST-RAP, as it did in those given GST (Table II). The increasing concentration of plasma HL in the GST-RAP group is consistent with reduced internalization of hepatic HL, which is mainly extracellular (3, 33). Indeed, as shown in Fig. 2B, enrichment of HL in the hepatocytic total endosome fraction was about 50% lower in animals given GST-RAP than in those given GST (p < 0.05). The effect of GST-RAP infusion on the concentration of LPL in plasma and its enrichment in endosomes was more pronounced than that observed for HL. As shown in Fig. 2C and Table III, the concentration of LPL in plasma was 2.5-fold higher in rats given GST-RAP than in those given GST, and LPL became undetectable in hepatocytic endosomes in the former group. Given the sensitivity of the ELISA for LPL, we estimate that endosomal enrichment of LPL was reduced by at least 95%. Enrichment of LPL in endosomes of control animals given GST (10-fold) was similar to that of HL in corresponding controls (8-fold), even though the mass of LPL in liver was less than 1% that of HL (compare Tables II and III). Livers contained 96.5% of the combined mass of LPL in liver + plasma in GST-infused control animals. This value was lower, 93.8%, in animals given GST-RAP (Table III). These results, taken together, suggest that HL is internalized into hepatocytes in part, and LPL is internalized almost entirely by a RAP-sensitive process or processes.

Effect of GST-RAP Upon Endosomal Enrichment of LRP and Other High Affinity Endocytic Receptors—The selected dose of
GST-RAP used in these experiments was sufficient to block completely the clearance of $a_2$-MG from plasma and, by inference, its endocytosis into hepatocytes by LRP (18). LRP is enriched in hepatocytic endosome fractions from untreated as well as estradiol-treated rats in a pattern consistent with extensive receptor recycling (37). GST-RAP is known to block similarly the endocytosis of all other high affinity ligands for LRP (38). We found no effect of the 30-min infusion of GST-RAP upon the mass of LRP in liver homogenates, as estimated by Western blotting (Fig. 3A). As shown in Fig. 3, A and B, however, endosomal enrichment was about 40% lower in animals given GST-RAP than in those given GST ($p < 0.05$). As in estradiol-treated rats (23), the LDL receptor was also comparably enriched in endosome fractions from untreated rats in a pattern consistent with receptor recycling.² GST-RAP infusion had no effect upon its endosomal enrichment, however. Furthermore, enrichment of other high affinity receptors studied previously in untreated or estradiol-treated rats (35, 39) was unaffected by GST-RAP infusion (Fig. 3B).

**Effect of GST-RAP Upon Endosomal Purity and Endosomal Trafficking Machinery.—**To determine whether GST-RAP affected the purity of our endosome fractions, we first addressed Golgi contamination by probing for GM130, a cis-Golgi marker (40). GM130 was only slightly enriched in endosomes from GST-infused animals (−3-fold over homogenate), and no difference was found in endosomes from animals given GST-RAP (data not shown). Enrichment of total cholesterol and triglycerides in endosomes was also unaffected by GST-RAP infusion (Table IV). Plasma concentrations of total cholesterol and triglycerides in blood samples taken at the end of the infusions were also comparable in animals given GST (cholesterol 57 ± 3.7 mg/dl and triglycerides 66 ± 7.4 mg/dl) and GST-RAP (cholesterol 49 ± 4.4 mg/dl and triglycerides 55 ± 6.4 mg/dl, $n = 6$).

To evaluate the effects of GST-RAP on other properties of the isolated endosomes, we also evaluated the endosomal enrichment of several components of the membrane traffic machinery associated with the endocytic pathway in rat hepatocytes. The enrichment values reported here (Fig. 4A) are somewhat lower than those we have found previously in estradiol-treated rats (39). No effect of GST-RAP infusion was found for two integral membrane proteins, cellubrevin and endobrevin, which are soluble N-ethylmaleimide-sensitive factor attachment protein receptors or for the low molecular weight GTPases, Rab5 and -11, which are peripheral membrane proteins. By contrast, we found a striking reduction by about 85% ($p < 0.01$) in the enrichment of EEA1, an early endosome marker that is abundant in endosome fractions from rat liver (39), in animals given GST-RAP (Fig. 4, A and B). This finding is in clear contrast with the lack of other evidence for an effect of GST-RAP on the general properties of our endosome preparation.

**TABLE III**

Concentration and % distribution of LPL in plasma and liver after 30-min infusions of GST or GST-RAP

|                | GST         | GST-RAP     |
|----------------|-------------|-------------|
| **Plasma LPL** | ng/ml       | %           | ng/ml       | %           |
| 1.0 ± 0.3      | 3.5 ± 0.9   | 2.4 ± 0.4   | 6.2 ± 0.8   |
| **Hepatic LPL**| ng/mg protein | %         | ng/mg protein | %         |
| 0.3 ± 0.0      | 96.5 ± 0.9  | 0.4 ± 0.0   | 93.8 ± 0.8  |

**TABLE IV**

Total cholesterol and triglycerides in hepatocytic endosomes after infusion of GST or GST-RAP

|                | GST         | GST-RAP     |
|----------------|-------------|-------------|
| **TC**         | fold enrichment over liver homogenate | 5.9 ± 1.3   | 5.0 ± 0.6   |
| **TG**         | fold enrichment over liver homogenate | 5.3 ± 0.9   | 6.3 ± 1.1   |

² M. Vergés and R. Havel, unpublished observations.

**DISCUSSION**

In this work, we have determined the effect of GST-RAP upon the concentration of HL and LPL in endosomes isolated from rat liver in order to evaluate the role of LRP in the endocytosis of these lipolytic enzymes. Our earlier ultrastructural studies have established the hepatocytic origin of three fractions (23, 32, 34). Two of these fractions are composed of lipoprotein-filled vesicles that we have characterized as early and late endosomes. The third tubular fraction, characterized as a receptor-recycling compartment, also contains the transcytotic polymeric immunoglobulin receptor, indicating broad involvement in receptor sorting (39). We have shown that HL undergoes endocytosis into hepatocytes, with a pattern of distribution among the three endosome fractions that is characteristic of ligands destined for degradation in lysosomes (23, 34, 35). At the time that we made these initial observations (16), it was generally thought that HL in liver is mainly or exclusively

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**FIG. 3.** Livers from the experiments shown in Fig. 2 were used to determine the fold enrichment of high affinity receptors in endosomes/homogenate. Receptor proteins were separated by SDS-PAGE and visualized by Western blotting as described under “Experimental Procedures.” A, representative Western blot showing reduced enrichment of LRP in endosomes from a rat given GST-RAP, as compared with a rat given GST. Liver homogenate (Hom), 50 μg of protein/lane; endosomes (End) 1 μg of protein/lane. B, bar graph showing that mean LRP mass was reduced by ~40% in rats given GST-RAP (*, $p < 0.05$), whereas that of other receptors was unaffected. LDLR, LDL receptor; plgR, polymeric immunoglobulin receptor; TfR, transferrin receptor; AGPR, asialoglycoprotein receptor; EGFR, epidermal growth factor receptor. All values are mean ± S.E. ($n = 6$).
hepatocytes and that the binding capacity of parenchymal cells.

Endocytosis occurs after migration of HL to coated pits located on HSPGs on hepatocytic microvilli. We therefore suggest that HL binds with abluminal surfaces of endothelial cells. In rats, the availability of HL is a RAP-sensitive process. LRP binds with the surface of freshly isolated rat hepatocytes, were, however, consistent with a more direct endocytic pathway involving LPL, HL may also enter hepatocytic endosomes via a complex pathway, involving transfer of endothelial HL to hepatocytic microvilli and then to coated pits. Our observations and those of Marteau et al. (43), who showed that HL is associated with the surface of freshly isolated rat hepatocytes, were, however, consistent with a more direct endocytic pathway involving hepatocytes alone. Subsequently, Bredereke et al. (44) showed that earlier work on the binding of HL to non-parenchymal rat liver cells resulted from contaminating “blebs” derived from hepatocytes as well as the capacity of parenchymal cells could account for almost all HL in rat liver. Bredereke et al. (44) also found, by immuno-gold electron microscopy of rat livers perfused with goat antibodies to rat HL, that HL is localized mainly in the space of Disse on hepatocytic microvilli, with only a minor fraction localized to the luminal side of endothelial cells (44). Previous major localization to endothelial cells was attributed possibly to nonspecific uptake of ferritin-coupled protein that binds GST-RAP with high affinity. We therefore proposed that HSPGs on human fibroblasts bind GST-RAP (49–51), and it has been suggested that HSPGs on human fibroblasts bind GST-RAP (14). Vassiliou and Stanley (52) showed that GST-RAP binds to a large number of low affinity sites on human fibroblasts in addition to its high affinity binding to LRP. Although binding of GST-RAP to the low affinity sites could be displaced by heparin, it was unaffected by treating the cells with heparinase or inhibiting sulfation of glycosaminoglycan chains of HSPGs (52). In our experiments, infusion of GST-RAP did not appreciably reduce the total hepatic mass of HL or LPL (Tables II and III). The increase in HL and LPL concentration in plasma produced by GST-RAP thus differs from the massive release of these enzymes from liver produced by the ionic action of heparin (3, 33). The progressive increase in plasma HL concentrations and the proportionately larger increase in plasma LPL during GST-RAP infusion can be explained by a reduced rate of endocytosis. These results confirm the observations of van Vliet et al., who found LRP to be the only liver membrane protein that binds GST-RAP with high affinity. We therefore conclude that GST-RAP reduces endocytosis of HL and LPL by specifically preventing their binding to LRP.

Infusion of GST-RAP into rats for 30 min essentially eliminated LPL from the isolated endosomes (>95% reduction in mass), indicating that LRP provides essentially the sole pathway for internalization of LPL into rat hepatocytes. By contrast, the 30-min infusion of GST-RAP reduced the endosomal concentration of HL by only 50%. Provided that the distribution of HL and LPL among endosomal compartments at the onset of the infusions is comparable, transfer to lysosomes during the 30-min infusions should be equally efficient. In estradiol-treated rats, transfer of LDL from the cell surface of hepatocytes to lysosomes requires about 30 min (32). In these rats, HL was mainly found in multivesicular bodies (Table I), the immediate prelysosomal compartment, which should accelerate transfer to lysosomes. These results suggest that, by contrast with LPL, HL may also enter hepatocytic endosomes via a RAP-insensitive pathway. Schoonderwoerd et al. (33) have proposed that binding of HL to rat liver includes both heparin-sensitive and heparin-resistant components. They provided evidence that perfusion of livers with heparin released a component or components in addition to HL that are required, in part, for binding of rat HL infused subsequently. Such components were not released by perfusion of livers with 0.3 M
NaCl, which, like heparin, released most HL from the liver. Subsequently, Breedveld et al. (53) isolated an HL-binding protein of 70 kDa in perfusates of rat liver, the identity of which was not determined. Our results with GST-RAP are consistent with the hypothesis that only HL bound to one of the distinct binding sites on hepatocytes has the capacity to interact with LRP.

Intravenously injected LPL is taken up efficiently by the liver but only slowly degraded (54). There is little information about the binding sites for LPL in liver, but it should be noted that Schoonderwoerd et al. (33) found no evidence for binding heterogeneity of LPL in their studies in perfused rat livers. Vilaro et al. (55) reported on the localization of HL and LPL in livers of rats infused with Intralipid to increase the hepatic uptake of LPL. By visualizing protein A-gold complexes with specific antibodies, they found similar localization of the enzymes in electron photomicrographs, at the luminal surface of endothelial cells, and within the space of Disse. Of note, no complexes were observed for LPL in rats not given Intralipid. The apparently complete inhibition of endocytosis of LPL by GST-RAP in our current studies strongly suggests that, like HL, LPL on liver surfaces is largely or entirely bound to hepatocytic microvilli in the neighborhood of LRP.

The distribution of HL and LPL activities between liver and blood plasma of rats has been studied by Peterson et al. (17). They found much more enzyme activity in the liver than in plasma, in general agreement with our measurements of the mass of the two enzymes. Our measurements also show that the mass of LPL in liver is only 0.4% that of HL (compare Tables II and III). As endosomal enrichment of LPL and HL was similar, we infer that the rate of endocytosis of LPL is likely to be 2 orders of magnitude lower than that of HL. In plasma, we found the concentration of LPL to be 1.4% that of HL. Although both enzymes have the capacity to exert important ligand-binding functions (7), these data suggest that HL has much larger potential for binding to chylomicron remnants and facilitating their endocytosis into hepatocytes via binding to LRP or the LDL receptor.

In the current work, we evaluated the effect of GST-RAP on several properties of our combined endosome fractions. GST-RAP did not affect the recovery of protein, an estimate of endosome mass. Endosome membranes are rich in cholesterol (23, 34), and in hepatocytes their contents are enriched in triglycerides contained in remnant lipoproteins (24). Infusion of GST-RAP had no effect on endosomal enrichment of these lipids. Enrichment over homogenate of several endocytic receptors, including LDLR, was also unaffected, consistent with no change in the purity of the fractions; however, enrichment of LRP was reduced by ~40% (Fig. 3). GST-RAP not only binds with high affinity to LRP but is also rapidly endocytosed by rat hepatocytes after intravenous injection (18), presumably together with LRP. GST-RAP, which binds to multiple sites on extracellular domains of LRP (38), thus appears to affect the rate of endocytosis or recycling of this receptor in rat hepatocytes. The step or steps in the itinerary of LRP that are altered by GST-RAP remain to be determined.

Although GST-RAP had no effect upon endosomal enrichment of LDLR, this receptor does bind GST-RAP with low affinity (18, 36). In human fibroblasts, the concentration of GST-RAP required to inhibit degradation of LDL by 50% is about 1 order of magnitude higher than that for α-MG (36). Because one-half of the dose of GST-RAP infused in our experiments inhibited hepatic uptake of α-MG (a high affinity ligand for LRP) by only about 50%, it is highly unlikely that the full dose used would have an appreciable effect upon uptake of LDLR ligands.

Infusion of GST-RAP also had no effect upon the enrichment of several protein components of the endosomal trafficking machinery, with one notable exception. Endosomal enrichment of EEA1 was strikingly reduced (Fig. 4). EEA1 is a cytoplasmic, coiled-coil protein of 162 kDa that is required for fusion of endosomes (56). Its C terminus contains a zinc finger motif (FYVE domain) that interacts with phosphatidylinositol 3-phosphate and an adjacent Rab5-binding region (57). Association of EEA1 with endosomes requires phosphorylation of phosphatidylinositol by its specific phosphatidylinositol 3-kinase (58). We have found EEA1 to be enriched more than 300-fold in early endosomes from livers of estradiol-treated rats, with somewhat lower enrichment in late endosomes and about 10-fold lower enrichment in recycling endosomes (39). In the current work, in which only a combined endosome fraction was isolated, we cannot exclude an effect of GST-RAP on the distribution of protein mass among the three subfractions, but this should have been apparent from changes in the enrichment of recycling receptors or other components of the trafficking machinery. Furthermore, as endocytosed GST-RAP should be located within the vesicular compartments of endosomes, it should not have direct access to a tethering molecule such as EEA1 or other related cytoplasmic proteins. The loss of EEA1 from endosomes can be explained, however, by an LRP-dependent activation of the p38 stress-activated protein kinase, which stimulates formation of a cytosolic complex between Rab5 and the guanyl nucleotide dissociation inhibitor (59). A recent study by Gardai et al. (60) found that clustering of LRP by extracellular ligands activates p38 in macrophages. In our experiments, a similar clustering of LRP and subsequent p38 activation may have been caused by the presence of multivalent aggregates in the GST-RAP preparation that was injected into the rats. Solubility of GST-RAP decreases at the high protein concentration necessary for intravenous injection into experimental animals.

In conclusion, our current data indicate that HL is partially and LPL almost exclusively taken up by endocytosis into rat hepatocytes via binding to LRP. This pathway likely leads to lysosomal degradation and has an important role in the turnover of these enzymes.

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