Mechanism of Activation and Functional Significance of the Lipolysis-Stimulated Receptor. Evidence for a Role as Chylomicron Remnant Receptor†

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ABSTRACT: In cultured human and rat cells, the lipolysis-stimulated receptor (LSR), when activated by free fatty acids (FFA), mediates the binding of apoprotein B- and apoprotein E-containing lipoproteins and their subsequent internalization and degradation. To better understand the physiological role of LSR, we developed a biochemical assay that optimizes both the activation and binding steps and, thus, allows the estimation of the number of LSR binding sites expressed in the livers of living animals. With this technique, a strong inverse correlation was found in rats between the apparent number of LSR binding sites in liver and the postprandial plasma triglyceride concentration ($r = -0.828$, $p < 0.001$, $n = 12$). No correlation existed between the number of LSR and plasma triglycerides measured in the same animals after 24 h of fasting. The same membrane binding assay was used to elucidate the mechanism by which FFA induce lipoprotein binding to LSR. The LSR activation step was mediated by direct interaction of FFA with LSR candidate proteins of apparent molecular masses of 115 and 90 kDa and occurred independently of the membrane lipid environment. The FFA-induced conformational shift that revealed the lipoprotein binding site remained fully reversible upon removal of the FFA. However, occupancy of the site by the apoprotein ligand stabilized the active form of LSR. Comparison of the effect of different FFA alone or in combination indicated that the same binding site is revealed by different FFA and that the length and saturation of the FFA monomeric carbon chain are critical in determining the potency of the FFA activating effect. We propose that the LSR pathway represents a limiting step for the cellular uptake of intestinally derived triglyceride-rich lipoproteins and speculate that FFA liberated by lipolysis initiate this process by altering the conformation of LSR to reveal the lipoprotein binding site.

Chylomicrons are assembled by the intestine to transport dietary triglycerides and cholesterol. Once in circulation, rapid hydrolysis of their triglyceride core leads to the formation of cholesterol-enriched chylomicron remnants (CMR),[1] which represent atherogenic particles (Zilversmit, 1979). Indeed, studies in transgenic mice that lack the CMR receptor ligand apoprotein (apo) E have clearly demonstrated that the inability to remove remnants from circulation leads to the development of severe aortic lesions in a species that is otherwise resistant to atherosclerosis (Zhang et al., 1992; Plump et al., 1992).

Considerable interest has thus focused on characterizing the receptors responsible for the removal of CMR by hepatocytes. One of these is the well-defined LDL receptor, which binds both apo B and apo E (Brown & Goldstein, 1986) and has been estimated to account for half of CMR removal (Choi et al., 1991). Indeed, CMR clearance is defective in homozygous Watanabe heritable hyperlipidemic rabbits, which lack functional LDL receptors (Bowler et al., 1991). However, the rate of CMR removal is normal in human subjects homozygous for familial hypercholesterolemia (FH), who present a defect in the LDL receptor (Rubinstein et al., 1990). It is therefore likely that besides the LDL receptor, at least one other receptor functions to remove CMR.

A significant breakthrough came with the molecular cloning of the LDL receptor-related protein (LRP) (Herz et al., 1988). This receptor bears significant structural homology with the LDL receptor (Herz et al., 1988) and mediates the internalization of βVLDL enriched with recombinant apo E (Kowal et al., 1990). The LRP was subsequently found to be identical to the α_2-macroglobulin receptor and to bind several ligands apparently not related to the lipoprotein system [for a review, see Williams et al. (1994)]. The extent of LRP's contribution to CMR clearance, however, has been questioned (van Berkel et al., 1994). It has also been hypothesized that proteoglycans mediate binding and internalization of lipoproteins either directly or through bridging with lipoprotein lipase (Eisenberg et al., 1992; Williams et al., 1992; Ji et al., 1993; Mulder et al., 1993). However, the relatively slow rate of internalization of cell surface proteoglycans (Eisenberg et al., 1992; Mulder et al., 1993)

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[1] Abbreviations: apo, apoprotein; BSA, bovine serum albumin; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; CMR, chylomicron remnants; FFA, free fatty acid(s); FH, familial hypercholesterolemia; LDL, low-density lipoproteins; LRP, LDL receptor-related protein; LSR, lipolysis-stimulated receptor; PBS, phosphate-buffered saline; RAP, receptor-associated protein; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; VLDL, very low-density lipoproteins.

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is more consistent with a role of sequestration of CMR at the cell surface than one of direct mediation of rapid cellular uptake of these particles.

An alternative mechanism for rapid internalization and degradation of CMR is provided by the lipolysis-stimulated receptor (LSR). The LSR, distinct from both the LDL receptor and the LRP, binds lipoproteins when activated by free fatty acids (FFA) (Bihain & Yen, 1992; Yen et al., 1994) and displays the greatest affinity for triglyceride-rich lipoproteins, i.e., those undergoing the most rapid lipolysis. That LSR might be a second CMR receptor is supported by its complete inhibition by lactoferrin (Yen at al., 1994), a milk protein that delays CMR clearance *in vivo* (Huettinger et al., 1988).

Recent studies by Willnow et al. (1994), using adenovirus-mediated gene transfer techniques in mice, established that clearance of CMR is inhibited by a 39 kDa protein referred to as receptor-associated protein (RAP). Although RAP binds to different members of the LDL receptor gene family, the strong inhibition of CMR clearance observed in mice lacking the LDL receptor (Willnow et al., 1994), together with the fact that neither gp330 nor the VLDL receptor is expressed in the liver (Kounnas et al., 1994; Takahashi et al., 1992), led these authors to conclude that the effect was mediated by the inhibition of LRP. Previous experiments showed that RAP at concentrations of up to 5 μg/mL had no significant effect on LSR activity, while fully inhibiting the LRP-mediated uptake and degradation of α_2-macroglobulin−methylamine (Yen et al., 1994). However, the concentrations of RAP reported by Willnow et al. to delay CMR clearance *in vivo* (20−200 μg/mL) do inhibit the activity of LSR (Troussard et al., 1995).

The first aim of the current study was to develop a biochemical assay for the estimation of the number of LSR binding sites expressed in purified plasma membranes from rat hepatocytes. By employing this technique, our goals were to define the functional role of LSR and to characterize the mechanism through which FFA activate the LSR. We report that a strong inverse correlation exists between the apparent number of LSR binding sites in the liver and the postprandial plasma triglyceride concentration. Data presented here also demonstrate that direct FFA interaction with LSR proteins causes a reversible shift in the conformation of the LSR that reveals a lipoprotein binding site.

EXPERIMENTAL PROCEDURES

Materials

Na^{125}I and [9,10-^{3}H(N)]oleic acid were purchased from Amersham and NEN, respectively (Les Ulis, France). Bovine serum albumin (BSA) (A2153), heparinase (H2519), heparitinase (H8891), chondroitinase (C2905), egg phosphatidylcholine (P4279), trypsin (T8003), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), and *n*-octyl glucoside were obtained from Sigma (St. Quentin Fallavier, France). FFA were obtained from Nu-Chek Prep Inc (Elysian, MN). Human lactoferrin (Serva) was purchased from Biowhittaker (Fontenay sous Bois, France). Enzymatic kits for the determination of FFA (Wako Chemicals) were purchased from Unipath SA (Dardilly, France), and those for phospholipids were obtained from BioMérieux (Marcy l'Etoile, France). Triglyceride (701912), cholesterol (1112350), and unesterified cholesterol (310328) reagents, standards, and control sera were purchased from Boehringer Mannheim (Meylan, France).

Methods

Animals. Male Sprague−Dawley rats (Janvier Breeding Center, Le Genest-St. Isle, France) were maintained at a temperature of 22 °C in an animal care facility equipped with a 12 h light/dark cycle. Animals were given water and regular chow (Aliment A04, Alimentation Rationelle, Villemoisson/Orge) *ad libitum*, except during fasting (24 h prior to blood sampling or overnight prior to liver isolation) when they received water only. For the isolation of livers, rats were anesthetized lightly with ether, and the livers were perfused through the portal vein with ice-cold saline solution (150 mM NaCl, 2 mM EDTA, pH 7.4) at 35 mL/min for 3 min. The livers were then immediately excised.

For the measurement of plasma lipid parameters, blood (400 μL) was drawn between 9:00 and 10:00 a.m. from the tail vein using a 26G needle and placed immediately into tubes kept on ice containing 2 mg of Na_2EDTA per tube. Plasma samples obtained by centrifugation (1800*g*, 10 min, 4 °C) were supplemented with aprotinin (0.14 IU/mL, final concentration) and kept at 4 °C. Lipid determinations were conducted within 24 h.

Preparation of Lipoproteins for Competition Experiments. VLDL (density (d) < 1.006 g/mL) and LDL (1.019 < d < 1.063 g/mL) were isolated from overnight-fasted human subjects (Havel et al., 1955). Chylomicrons were prepared by ultracentrifugation (30000*g*, 1 h, 10 °C, Beckman Ti50.2 rotor) of plasma drawn from the same subjects on the same day, 2 h after a breakfast consisting of three croissants with butter and jam and 250 mL of whole milk. Contaminating albumin was removed from the chylomicron fractions by incubation for 30 min at room temperature with an equivalent volume of swollen Blue Sepharose CL-6B gel (Pharmacia Biotech, Guyancourt, France). After this treatment, albumin represented less than 2% of total protein, as assessed by image analysis (ImageMaster, Pharmacia) of Coomassie Blue-stained 4−18% SDS−PAGE gradient gels. All lipoproteins were stored in the dark at 4 °C under N_2 and used within 3 days of their isolation.

Isolation and Radiolabeling of LDL. Human LDL (1.025 < d < 1.055 g/mL) were isolated by sequential ultracentrifugation of fresh plasma obtained from the local blood bank. The LDL preparations were stored in the dark at 4 °C under N_2 and were used within 2 weeks of their isolation. LDL were radioiodinated by the modified McFarlane's method (Bilheimer et al., 1972), as previously described (Goldstein et al., 1983). [^{125}I]LDL were used within a week of preparation and were filtered (0.2 μm filter, Gelman, Ann Arbor, MI) immediately prior to use. Specific activities of [^{125}I]LDL preparations ranged between 96 and 432 cpm/ng of protein.

Rat Hepatocyte Plasma Membrane Isolation. Hepatocyte plasma membranes were prepared from the livers of fasted rats weighing between 125 and 150 g, as previously described (Bartles & Hubbard, 1990). All buffers used in the isolation procedure contained EDTA (2 mM) and were supplemented with 0.01 vol of the following inhibitor cocktail: leupeptin (1 mg/mL), benzamidine (10 mg/mL), and sodium azide (300 mM). Plasma membranes were stored in the dark at 4 °C

Table 1: Enrichment of Organelle Markers during the Isolation of Hepatocyte Plasma Membranes

purification step	enrichment (-fold)			
	5′-nucleotidase (plasma membrane)	glucose-6-phosphatase (microsomes)	cytochrome *c* oxidase (mitochondria)	β-galactosidase (lysosomes)
liver homogenate	1.0	1.0	1.0	1.0
supernatant after first spin (280*g*, 5 min)	1.9	2.3	1.2	1.3
pellet after second spin (1500*g*, 10 min)	3.5	2.8	0.9	0.7
plasma membrane preparation (by flotation, $1.03 < d < 1.18$ g/mL)	12.1	0.8	0.5	1.0

under N_2 in the presence of the inhibitor cocktail and were used within 7 days of their preparation.

Verification of the purity of the plasma membrane preparations was routinely conducted by measurement of different membrane markers in the whole liver homogenate and in the isolated fraction. Activity of the plasma membrane marker, 5′-nucleotidase, was measured by using a kit obtained from Sigma Diagnostics. Assays for glucose-6-phosphatase (de Duve et al., 1955), cytochrome *c* oxidase (Storrie & Madden, 1990), and β-galactosidase (Storrie & Madden, 1990) activities were used to determine the relative contents of microsomal, mitochondrial, and lysosomal membranes, respectively. Table 1 shows the enrichment of plasma membranes compared to that of the other types of membranes, obtained at the different steps of a typical purification procedure.

Assay for the Measurement of FFA-Induced Binding of [^{125}I]LDL. To quantify LSR activity in isolated hepatocyte plasma membranes, a technique was developed to optimize [^{125}I]LDL binding induced by FFA. All buffers were prepared without Ca^{2+} and were supplemented with 2 mM EDTA to suppress the activities of both the LDL receptor (Goldstein et al., 1977) and the LRP (Herz et al., 1988). Immediately prior to use, membranes were diluted to a concentration of 1 mg of protein/mL with 0.1 M phosphate buffer containing 350 mM NaCl and 2 mM EDTA (pH 8.0) (buffer A) and sonicated (Bioblock Scientific Vibracell, power 2.5, 25% pulse, 30 s). Aliquots of membrane were incubated in microfuge tubes at 37 °C for 30 min in the absence or presence of FFA (adjusted to a final volume of 250 μL with buffer A). For each sample, the indicated amount of FFA, from stock solutions of 25 or 37.5 mM in isopropyl alcohol, was rapidly injected (≤10 μL) using a Hamilton microsyringe. At these concentrations, isopropyl alcohol alone had no effect on [^{125}I]LDL binding to membranes. Optimal delivery of FFA to the sample required that the tip of the syringe be placed below the air−liquid interface and that the sample be briefly agitated immediately after injection. To remove FFA not bound to plasma membranes, the samples were centrifuged (35000*g*, 15 min, 12 °C, Sorvall F28/Micro rotor), the supernatants were removed, and the pellets were resuspended into 250 μL of buffer A by brief sonication (power 1.0, 90% pulse, 5 s). This washing procedure was repeated twice unless otherwise indicated. After the final wash the membrane pellets were resuspended into 210 μL of buffer A. Aliquots of [^{125}I]LDL were added from freshly prepared dilutions of the stock solution in buffer A to give the indicated concentrations of [^{125}I]LDL in a final volume of 250 μL. The samples were then incubated at 37 °C for 60 min. Membrane-bound [^{125}I]-LDL was separated from unbound [^{125}I]LDL by layering a 200 μL aliquot over a 600 μL cushion of 5% (w/v) BSA in buffer A and centrifuging (35000*g*, 20 min, 4 °C, Sorvall F28/Micro rotor) to pellet the membranes. After careful aspiration of the supernatants, the bottoms of the tubes were cut and counted for ^{125}I in a Pharmacia 1470 Wizard γ-counter.

Solubilization of Membrane Protein and Reconstitution into Artificial Membranes with Phospholipid. Aliquots of isolated membranes (10 mg of protein) were pipetted into polyallomer microfuge tubes and centrifuged in a Beckman Optima TL ultracentrifuge (125000*g*, 10 min, 4 °C, TLA45 rotor). After removal of the supernatant, each pellet was resuspended with a 21G needle into 1 mL of buffer A containing 40 mM *n*-octyl glucoside and inhibitor cocktail. The suspensions were sonicated (power 2.5, 15% pulse, 30 s), transferred to thick-walled polycarbonate Beckman TLA120.2 tubes, and centrifuged (400000*g*, 30 min, 4 °C, TLA120.2 rotor). The resulting clear supernatants of soluble protein (3−4 mg/mL) were kept at 4 °C under N_2 for up to 2 days without significant loss of binding activity. These preparations contained less than 0.1 mg/mL phospholipid.

Protein/phospholipid liposomes were prepared by using the phosphatidylcholine/acetone precipitation method described by Schneider et al. (1980). Suspensions of egg phosphatidylcholine liposomes were prepared in buffer A (1.8−2.0 mg of phospholipid/mL) and stored in aliquots at 4 °C under N_2 for up to 6 weeks. To incorporate solubilized protein into liposomes, aliquots of 5 mg of solubilized protein were supplemented with different amounts of phosphatidylcholine liposome suspension. The volumes of the samples were adjusted to 7 mL with 0.1 M phosphate buffer (pH 8.0) containing 600 mM NaCl and 2 mM EDTA. This large volume ensured the dilution of *n*-octyl glucoside to less than 12 mM, i.e., below its critical micellar concentration. Ice-cold acetone [4.2 mL to achieve a 37.5% (v/v) acetone solution] was then added rapidly while the samples were vigorously vortex mixed. After incubation for 2 min on ice, the resulting precipitates were sedimented by centrifugation (40000*g*, 10 min, 4 °C, Beckman SW41 rotor). The supernatants were carefully removed by aspiration, and the protein/phospholipid pellets were resuspended into 1 mL of buffer A by sonication (power 2.0, 20% pulse, 30 s).

Preparation of LSR-Enriched Solubilized Protein Fraction. Larger quantities of soluble protein were obtained as described earlier, except that CHAPS was used instead of *n*-octyl glucoside. Aliquots of membranes (40 mg of protein) were pipetted into thick-walled polycarbonate tubes and centrifuged in a Beckman Ti75 rotor (120000*g*, 20 min, 4 °C). After removal of the supernatant, each pellet was resuspended with a 21G needle into 5 mL of buffer B (20 mM Tris, 150 mM NaCl, 2 mM EDTA, 13 mM CHAPS, and inhibitor cocktail, pH 8.0). Membrane−detergent mixtures were sonicated (power 2.5, 25% pulse, 30 s), incubated at 4 °C for 30 min, and then recentrifuged (200000*g*, 2 h, 4 °C). The supernatants containing the soluble protein were

pooled and filtered through a 0.2 μm filter. Ninety milligrams of protein in 30 mL was applied at a rate of 0.2 mL/min to a Pharmacia Source Q anion-exchange column (2.0 × 9.2 cm) equilibrated with buffer B at room temperature. The column was washed with 60 mL of buffer B at the same flow rate, and then the proteins were eluted with a 50 mL linear gradient of 150 to 900 mM NaCl in buffer B at a flow rate of 0.30 mL/min. Fractions of 1.7 mL were collected, immediately placed on ice, and assayed for protein mass and LSR activity. Eluted fractions enriched with LSR were detected by ligand blotting.

Ligand Blotting. The LSR-enriched fraction from solubilized membrane protein was loaded directly onto an SDS–PAGE gradient gel (4–12%, 1.0 mm thickness, 75 μg of protein/lane, 50 V, 18 h). After separation, the proteins were transferred to nitrocellulose (0.45 μm nitrocellulose membrane, Schleicher and Schuell; transferred by Trans Blot SD Electrophoretic Transfer Cell, Bio-Rad, 18 V, 25 min). The nitrocellulose strips were blocked using phosphate-buffered saline (PBS) containing 3% (w/v) BSA (30 min, room temperature), washed four times with PBS over a period of 40 min, and then incubated at 37 °C for 30 min in buffer A with or without oleate. After they were washed twice with buffer A, the nitrocellulose membrane strips were incubated at 37 °C for 30 min with or without 5mg/mL lactoferrin. After an additional wash with buffer A, the strips were incubated at 37 °C for 60 min in buffer A containing 20 μg/mL [^{125}I]LDL, and finally washed repeatedly with PBS containing 0.5% (v/v) Triton-X100 until the ^{125}I radioactivity in the wash was <200 cpm/mL. [^{125}I]LDL bound to the nitrocellulose membranes were revealed by overnight exposure to a phosphor screen and subsequent image analysis (Molecular Dynamics PhosphorImager SF).

Protein and Lipid Determinations. Protein contents of lipoproteins and membranes were measured by Markwell's modification of Lowry's procedure (Markwell et al., 1981), using BSA as standard. Lipid analyses were determined enzymatically using colorimetric kits (see Materials).

Statistical Analyses. Statistical significance of the difference between two means was determined by the two-tailed Student's *t*-test for unpaired samples. Regression analyses were performed using Pearson's correlation coefficient. All data are expressed as mean ± standard deviation (SD).

RESULTS

Effect of Different FFA on [^{125}I]LDL Binding to Rat Hepatocyte Plasma Membranes. We first tested the effect of preincubation of rat liver plasma membranes with increasing concentrations of different FFA on the binding of [^{125}I]-LDL. The results in Figure 1 show that differences existed in the ability of the FFA to induce binding of [^{125}I]LDL to the membranes. Of the unsaturated fatty acids (panel A), oleate demonstrated the strongest response; a shorter chain length or an increased number of double bonds reduced the potency of the FFA. For the saturated fatty acids (panel B), palmitate induced [^{125}I]LDL binding by up to 79% of the stimulatory effect achieved with oleate, while saturated FFA of longer or shorter chain lengths were less efficient. It is interesting to note that, of the FFA tested, the strongest responses were mediated by two of the most abundant FFA in rat plasma, i.e., oleate and palmitate (Polette et al., 1992). Combinations of these two FFA were tested to determine

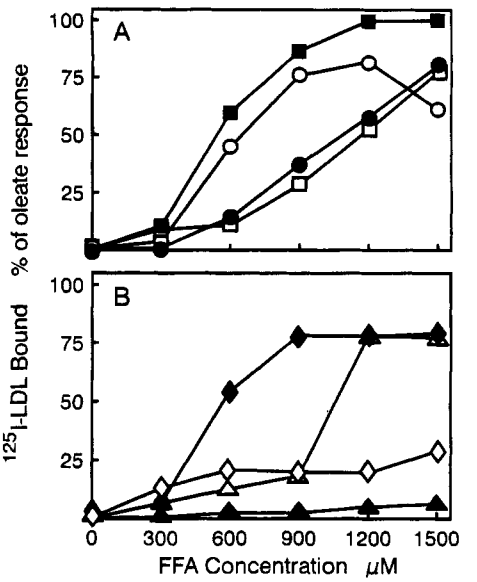

FIGURE 1: Comparison of the effect of different FFA on LSR activation. Aliquots of hepatocyte plasma membranes (100 μg of protein or the equivalent phospholipid amount of trypsin-treated membranes) were incubated at 37 °C for 30 min with increasing concentrations of the following FFA: (A) oleate (■), linoleate (○), γ-linolenate (□), and palmitoleate (●); (B) laurate (▲), myristate (△), palmitate (◆), and stearate (◇). After this, the membranes were washed three times with 0.1 M phosphate buffer (pH 8.0) containing 350 mM NaCl and 2 mM EDTA (buffer A) and incubated further at 37 °C for 60 min with 40 μg/mL [^{125}I]LDL (specific activity, 183 cpm/ng). Membrane-bound [^{125}I]LDL was measured by sedimenting the membranes through a 5% (w/v) BSA cushion, cutting the bottom of the tube, and counting the radioactivity in the pellet. [^{125}I]LDL binding was calculated by subtraction of values obtained with trypsinized membranes from those obtained with native membranes. Each point represents the mean of duplicate determinations, expressed as the percent of [^{125}I]LDL bound after incubation with oleate at 1500 μM.

whether their effects on [^{125}I]LDL binding were additive. Supplementation of palmitate to membranes preincubated with concentrations of oleate that achieved maximal binding was unable to further enhance lipoprotein binding. However, supplementation of oleate to membranes preincubated with palmitate at concentrations achieving maximal effect did increase [^{125}I]LDL binding by about 20%, i.e., up to the maximal binding of [^{125}I]LDL observed with oleate alone (data not shown). These data were interpreted to indicate that the same lipoprotein binding site is revealed by preincubation of membranes with either oleate or palmitate. For reasons that remain unclear, the latter appeared unable to recruit all potential binding sites (Figure 1). We therefore chose to use oleate as the probe to characterize the mechanism of this activation.

Effect of Phospholipid on Oleate-Induced [^{125}I]LDL Binding. Data in Figure 1 also showed that the dose–response curves obtained with oleate, palmitate, linoleate, and myristate appeared sigmoidal, with little or no increase in [^{125}I]LDL binding observed at low FFA concentrations (≤300 μM). We speculated that this lag phase resulted from the membrane phospholipid entrapping the FFA and thereby preventing the induction of [^{125}I]LDL binding. To test this, membrane protein was solubilized from its lipid environment using 40 mM *n*-octyl glucoside and reconstituted into artificial membranes with different amounts of egg phos-

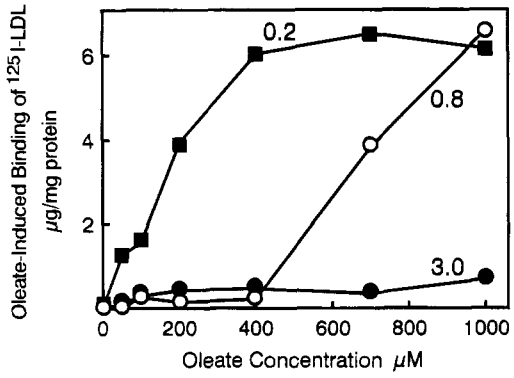

FIGURE 2: Effect of phospholipid mass on oleate-induced [^{125}I]-LDL binding. Proteins were solubilized from membranes using 40 mM *n*-octyl glucoside and incorporated into different amounts of egg phosphatidylcholine liposomes by acetone precipitation. Phospholipid and protein masses were remeasured in the resuspended precipitates and used to calculate the mass ratios of phospholipid to protein indicated: 0.2 (■), 0.8 (○), and 3.0 (●). Aliquots (100 μg of protein) of these reconstituted membranes were incubated at 37 °C for 30 min with the indicated concentrations of oleate, washed, and then further incubated at 37 °C for 60 min with 40 μg/mL [^{125}I]LDL (specific activity, 96 cpm/ng). Bound [^{125}I]LDL was measured by sedimenting the membranes through a 5% (w/v) BSA cushion, cutting the bottom of the tube, and counting the radioactivity in the pellet. Oleate-induced [^{125}I]LDL binding was calculated by subtraction of values obtained from parallel incubations conducted in the absence of oleate. Each point represents the mean of duplicate determinations.

phatidylcholine.[2] Conditions were chosen to vary the relative content of phospholipid, while maintaining the same amount of protein. Oleate dose−response curves were then obtained using the different reconstituted membranes. As shown in Figure 2, the mass of phospholipid relative to that of protein influenced the concentration of oleate required to induce [^{125}I]-LDL binding. With low amounts of phospholipid, i.e., a phospholipid to protein mass ratio of 0.2, oleate-induced [^{125}I]-LDL binding reached saturation at a concentration of 400 μM oleate. Under these conditions, the binding of [^{125}I]LDL was increased by oleate concentrations of as low as 50 μM. In contrast, when more phospholipid was present, i.e., a phospholipid to protein ratio of 0.8, oleate at concentrations of up to 400 μM had little to no effect on [^{125}I]LDL binding. Even greater amounts of phospholipid, i.e., a phospholipid to protein ratio of 3.0, prevented oleate-induced binding of [^{125}I]LDL at concentrations of oleate as high as 1000 μM. Phospholipid therefore appeared to act as a sink for FFA and prevented the latter from inducing lipoprotein binding.

Kinetics and Lipoprotein Binding Specificity of the Oleate-Induced Binding Site in Rat Hepatocyte Plasma Membranes. LDL binding curves obtained using plasma membranes preincubated either without oleate or with 1000 μM oleate are shown in panel A of Figure 3. The stimulatory effect of oleate increased the apparent number of LDL binding sites by more than 18-fold. Under such conditions, maximal [^{125}I]-LDL binding to plasma membranes was estimated at 49 μg of [^{125}I]LDL/mg of protein (panel B). Half-maximum binding occurred at a concentration of 23 μg of [^{125}I]LDL/mL.

[2] In the absence of detergent, oleate-induced binding activity could not be extracted from sonicated hepatocyte membranes. These data are consistent with the lipoprotein binding site being due to an intrinsic membrane protein.

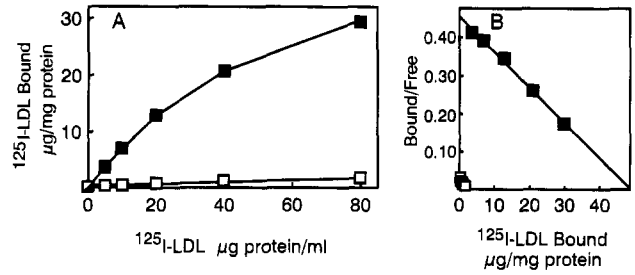

FIGURE 3: [^{125}I]LDL binding to rat hepatocyte plasma membranes as a function of [^{125}I]LDL concentration (A) and after transformation by Scatchard analysis (B). Aliquots of plasma membranes (100 μg of protein) were incubated at 37 °C for 30 min in the absence (□) or presence (■) of 1000 μM oleate. After washing, the membranes were incubated further at 37 °C for 60 min with the indicated concentrations of [^{125}I]LDL (specific activity, 397 cpm/ng). Bound [^{125}I]LDL was measured by sedimenting the membranes through a 5% (w/v) BSA cushion, cutting the bottom of the tube, and counting the radioactivity in the pellet. Each point represents the mean of triplicate determinations.

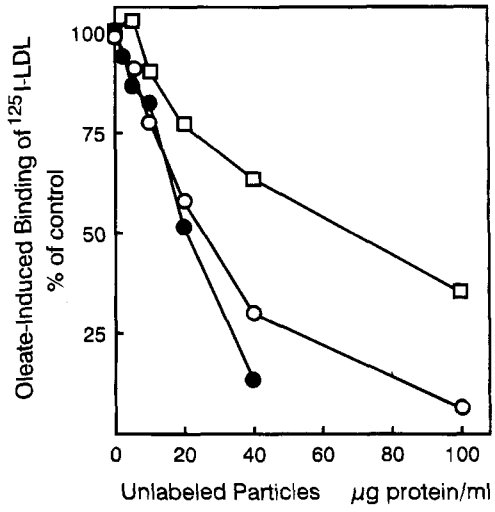

FIGURE 4: Competition of unlabeled lipoproteins with [^{125}I]LDL for binding to the oleate-induced binding site in hepatocyte plasma membranes. Aliquots of membranes (50 μg of protein) were incubated at 37 °C for 30 min in the presence of 1000 μM oleate. After washing, the samples were incubated at 37 °C for 60 min with 40 μg/mL [^{125}I]LDL (specific activity, 204 cpm/ng) and the indicated concentrations of unlabeled lipoproteins [chylomicrons (●), VLDL (○), LDL (□)]. Values for oleate-induced [^{125}I]LDL binding were obtained as described for Figure 2 and are expressed as a percent of the mean value obtained in the absence of unlabeled lipoprotein (19.7 ± 2.2 μg of [^{125}I]LDL per mg of protein, $n = 6$). All other points represent the means of duplicate determinations.

Figure 4 shows that, similar to what was observed in intact cells (Yen et al., 1994), chylomicrons and VLDL competed more efficiently for binding to hepatocyte plasma membrane LSR than did LDL. Although rat hepatocyte LSR showed greater affinity for triglyceride-rich lipoproteins, technical considerations led us to use LDL in subsequent analyses. First, LDL contains only a single nonexchangeable protein, the 550 kDa apo B, and second, during iodination of triglyceride-rich lipoproteins, unlike that of LDL, significant amounts of the radiolabel (up to one-fourth) become incorporated into the lipid moiety, which considerably increases the value of nonspecific binding.

The Apparent Number of LSR on Hepatocyte Plasma Membrane Correlates with the Nonfasting Plasma Triglyceride Concentration. We next sought to determine whether

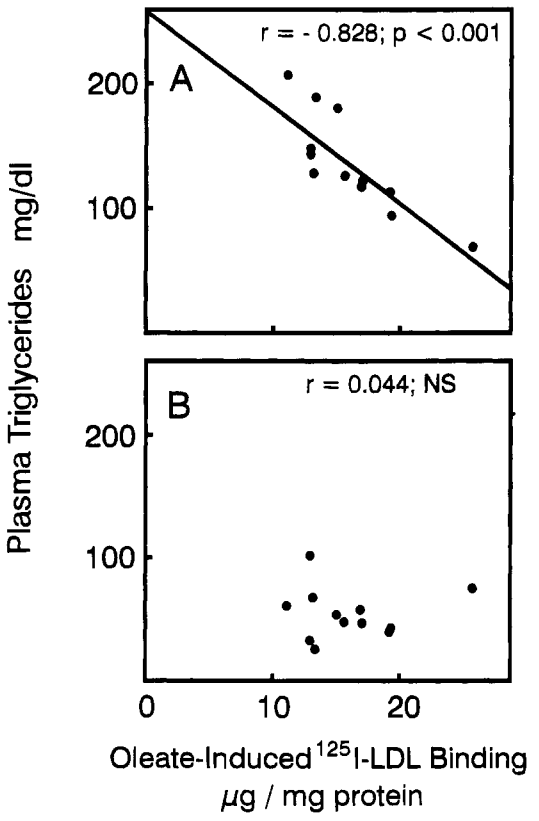

FIGURE 5: Correlation between hepatocyte plasma membrane oleate-induced [^{125}I]LDL binding and plasma triglyceride concentrations under fed (A) and fasted (B) conditions. Fasted and nonfasted plasma samples from 12 rats were obtained each on two separate occasion during the week prior to sacrifice by drawing blood from the tail vein; each triglyceride value represents the mean of the two determinations. Hepatocyte plasma membranes from individual livers were prepared as described in Methods. Aliquots of membranes (100 μg of protein) were incubated at 37 °C for 30 min in the presence of 1000 μM oleate. After washing, the samples were incubated at 37 °C for 60 min with 40μg/mL of [^{125}I]LDL (specific activity, 432 cpm/ng). Oleate-induced binding of [^{125}I]-LDL was calculated as described for Figure 2; each value represents the mean of triplicate determinations.

liver LSR might have functional relevance *in vivo*. In a series of randomly selected rats, we obtained an estimate of the number of LSR expressed on the hepatocyte plasma membrane and compared this number with the concentrations of cholesterol and triglycerides measured on plasma obtained in fasted and fed states. The results of this study revealed that a strong, inverse, and linear correlation existed between the apparent number of LSR and the plasma triglyceride concentration measured in the fed state (Figure 5A). In contrast, there was no correlation between apparent LSR number and plasma triglycerides measured in the same animals after 24 h of fasting (Figure 5B). These data suggested that the level of postprandial triglyceride-rich lipoproteins is related to the number of hepatocyte plasma membrane LSR binding sites. Further linear and nonlinear analyses failed to reveal any link between the apparent number of LSR and plasma cholesterol concentrations (data not shown).

To rule out the possibility that the link between the apparent number of LSR and plasma triglycerides in the fed state resulted from the interference of the latter with the membrane isolation procedure, we tested for but found no correlation between plasma triglycerides and the plasma membrane marker, 5′-nucleotidase (data not shown). Another possible explanation for the inverse correlation is that different levels of triglyceride-rich lipoproteins *in vivo* might be expected to lead to a difference in occupancy of LSR binding sites and thereby alter the apparent number of sites detected by our assay. To test this, one group of rats was allowed to feed while a second group was fasted prior to plasma membrane preparation. At the time of sacrifice, plasma triglycerides in the fed group were significantly higher than in the fasted group [127 ± 34 (n = 6) vs 69 ± 34 mg/dL (n = 5), respectively; $p < 0.02$]. However, the apparent number of oleate-induced binding sites was not significantly different between the groups [6.4 ± 0.3 vs 6.2 ± 0.6 μg of [^{125}I]LDL bound/mg of protein, respectively, using 800 μM oleate and 40 μg of [^{125}I]LDL/mL, 250 cpm/ng]. It is also unlikely that LSR engaged in the process of lipoprotein uptake at the time of liver isolation would retain their bound lipoprotein during the membrane purification procedure. Indeed, more than 50% of LSR-bound LDL are released by a single sonication (power 1.0, 90% pulse, 5 s) of plasma membranes. Between the time of isolation and the time of incubation with [^{125}I]LDL, the membranes were subjected to five sonications of similar or greater intensity. It thus appeared that our assay remained unaffected by potential LSR site occupation with endogenous lipoproteins at the time of sacrifice.

The notion that the apparent number of LSR is inversely associated with nonfasting plasma triglyceride concentration was further strengthened by the observation of a similar correlation achieved by using a different method of membrane preparation. This method (Belcher et al., 1987), based on sedimentation rather than flotation, yielded a lower degree of purification of plasma membranes (2−3-fold enrichment of 5′-nucleotidase activity). Nevertheless, with this second series of animals, an inverse and linear correlation between apparent LSR number and non-fasting plasma triglycerides was also observed ($r = -0.802$, $p < 0.01$, $n = 8$; data not shown).

These observations suggest that LSR might represent an important step in triglyceride-rich lipoprotein metabolism. Two possibilities have to be considered: either LSR represents a rate-limiting step in the overall process of triglyceride removal or LSR expression inversely adapts to the plasma triglyceride level. Further insight into the LSR itself is required to provide a better understanding of this regulation. As a first step toward this end, we sought to characterize the mechanism of LSR activation by FFA.

Mechanism of LSR Activation. (A) Protein and Not Proteoglycan Is Responsible for LSR Activity on Plasma Membranes. Several recent reports have argued for a role of heparan sulfate proteoglycans in lipoprotein removal (Eisenberg et al., 1992; Williams et al., 1992; Ji et al., 1993; Mulder et al., 1993). We thus tested whether proteoglycans influenced FFA-induced lipoprotein binding to LSR. Prior to measurement of binding activity, plasma membranes were incubated at 37 °C for periods of up to 1 h with a combination of heparinase and heparitinase or with chondroitinase. Figure 6 shows that, as compared to controls, incubation of membranes with either the heparinase/heparitinase mixture or with chondroitinase did not significantly modify [^{125}I]LDL binding to LSR. In contrast, parallel incubations of membranes with trypsin led to the complete

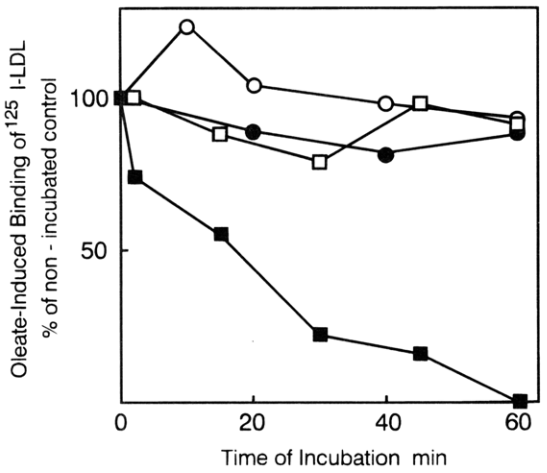

FIGURE 6: Effect of pretreatment of plasma membranes with a heparinase/ heparitinase combination, chondroitinase, or trypsin on oleate-induced [^{125}I]LDL binding. Membranes were first washed by two cycles of centrifugation (35000*g*, 10 min, 4 °C) and resuspension in PBS by consecutive passage through 21G and 26G needles to remove the protease inhibitor cocktail used during storage. Aliquots of membranes (500 μg of protein) were then made to 250 μL with PBS (□) or the same buffer supplemented with 10 units/mL each of heparinase and heparitinase (○), 10 units/mL chondroitinase (●), or 0.5 mg/mL trypsin (■). After brief sonication (power 1.0, 90% pulse, 5 s), the samples were incubated at 37 °C for the indicated times. The reactions were stopped by placing them on ice, adding aprotinin (0.14 units/mL), and washing by two cycles of centrifugation (35000*g*, 10 min, 4 °C) and resuspension into buffer A by sonication (power 1.0, 90% pulse, 5 s). Protein concentrations in the nontrypsinized samples were remeasured to compensate for losses during pretreatment. Aliquots containing 100 μg of protein, or for the trypsinized membranes an amount of phospholipid equivalent to that associated with 100 μg of protein in control membranes, were incubated at 37 °C for 30 min in the presence of 800 μM oleate. After this, the samples were washed and then further incubated at 37 °C for 60 min with 10 μg/mL [^{125}I]LDL (specific activity, 174 cpm/ng). Values for oleate-induced [^{125}I]LDL binding were calculated as described for Figure 2 and are expressed as a percent of the respective values obtained at time = 0 min (control = 1.8 μg/mg protein; heparinases = 1.6 μg/mg; chondroitinase = 2.0 μg/mg; trypsin = 1.8 μg/mg). Each value represents the mean of duplicate determinations.

loss of LSR activity. Control experiments showed that incubations of endothelial cells with the same preparation of heparinase/heparitinase efficiently degraded heparan sulfate at the cell surface; this markedly reduced the capacity of VLDL to bind to these cells (Yen et al., unpublished results). Thus, rat liver plasma membrane LSR activity is due to a protein and is not dependent on glycosaminoglycans.

The inactivation of LSR by trypsin treatment provided the means to test *in vivo* that LSR is expressed on the external surface of the plasma membrane. Rat livers were perfused with trypsin *in situ* to degrade those surface proteins directly in contact with the circulation. As shown in Figure 7, the presence of trypsin in the perfusate reduced by 80% the maximum number of oleate-induced [^{125}I]LDL binding sites on the isolated plasma membranes. This experiment, however, cannot exclude that part of the trypsin effect resulted from potential disruption of liver cells.

(B) LSR Activation Is Reversible When the Binding Site Is Unoccupied. We next tested whether the activation of LSR by FFA is a reversible process. Albumin provided the means to remove FFA from membranes after the FFA activation step (Bihain et al., 1989; Bihain & Yen, 1992).

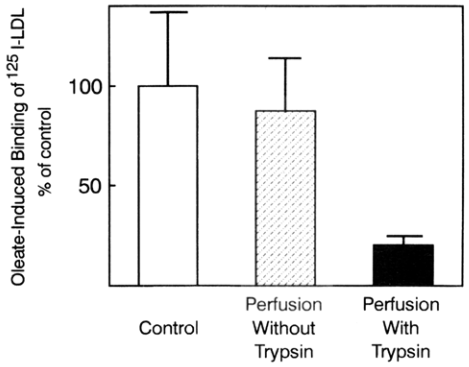

FIGURE 7: Effect of liver perfusion with trypsin on LSR activity in the isolated hepatocyte plasma membranes. Rat livers were perfused *in situ* with ice-cold saline solution (150 mM NaCl, 2 mM EDTA, pH 7.4) (35 mL/min for 3 min). One set of livers was excised directly (open bar). Two other sets were perfused at 37 °C for 10 min at 5 mL/min either with saline solution (hatched bar) or with the same solution containing 0.5 mg/mL trypsin (solid bar). These two sets of livers were then further perfused at 4 °C with rat plasma (50% (v/v) in saline solution) for 2 min at 5 mL/min to inactivate the trypsin and finally at the same temperature with saline solution for 2 min at 35 mL/min. After this, the livers were excised, and plasma membranes were prepared as described in Methods. Aliquots of 100 μg of protein, or for the trypsinized membranes an amount of phospholipid equivalent to that associated with 100 μg of protein in membranes obtained after perfusion without trypsin, were incubated at 37 °C for 30 min in the presence of 1000 μM oleate. After they were washed with buffer A, the samples were incubated further at 37 °C for 60 min with 40 μg/mL [^{125}I]LDL (specific activity, 113cpm/ng), and the amount of [^{125}I]LDL bound was determined. Values for oleate-induced [^{125}I]LDL binding were calculated as described for Figure 2 and are expressed as the percent of the mean of control values (10.7 μg/mg protein). Each bar shows the mean + SD of results obtained from four individual rats.

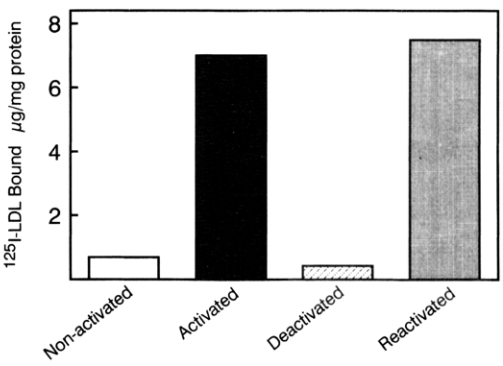

FIGURE 8: Reversibility of LSR activation by FFA. Four sets of membrane aliquots containing 100 μg of protein were prepared. The first set was incubated at 37 °C for 30 min without oleate, washed three times with buffer A, and again incubated at 37 °C for 30 min without oleate (open bar). The second set was incubated at 37 °C for 30 min in the presence of 800 μM oleate in the first incubation, washed three times with buffer A, and then incubated at 37 °C for 30 min without oleate (solid bar). The third set was incubated at 37 °C for 30 min with 800 μM oleate, washed twice with buffer A containing 1% (w/v) BSA and once with buffer A, and then reincubated in the absence of oleate (hatched bar). The fourth set was incubated at 37 °C for 30 min with 800 μM oleate, washed twice with buffer containing 1% (w/v) BSA and once with buffer A, and then reincubated with 800 μM oleate (gray bar). After this, all membrane samples were washed with buffer A and incubated at 37 °C for 60 min with 40 μg/mL [^{125}I]LDL (specific activity, 123 cpm/ng), and the amount of [^{125}I]LDL bound was determined as described for Figure 3. Each point represents the mean of duplicate determinations.

Figure 8 shows that when plasma membranes containing oleate-activated LSR were washed with albumin prior to the [^{125}I]LDL binding step, the amount of [^{125}I]LDL bound was at the same level as that bound to nonactivated membranes.[3] These data were consistent with the activated LSR being deactivated by removal of FFA. The question then arose concerning whether LSR activity could be restored after the membranes were washed with albumin. The fourth bar of Figure 8 shows that reincubation of albumin-washed membranes with oleate led to the same level of [^{125}I]LDL binding as that observed after a single exposure to oleate. Exposure of plasma membranes to two consecutive incubations with oleate, but without albumin washing did not further increase the level of LSR activation (data not shown).

We then examined whether removal of FFA after formation of the LSR−LDL complex leads to dissociation of the ligand. To test this, aliquots of membranes containing 100 μg of protein were incubated with 800 μM oleate for 30 min at 37 °C to activate the receptor, washed three times with buffer A, and then reincubated with [^{125}I]LDL (10 μg of protein/mL, 231 cpm/ng) for 60 min at 37 °C. After this, PBS containing 6% (w/v) BSA was added to achieve a final concentration of 1% (w/v) BSA, and the incubation mixtures were further incubated at 37 °C for 60 min. In these experiments, albumin was found unable to significantly decrease [^{125}I]LDL binding compared to incubations conducted without albumin [1.44 ± 0.12 μg of [^{125}I]LDL bound/mg of protein after incubation with albumin (n = 3) compared to 1.70 ± 0.31 μg of [^{125}I]LDL bound/mg protein after incubation with PBS alone (n = 3)]. These data indicated that exposure of the LSR−LDL complex to albumin failed to cause its dissociation. This could have resulted from a stabilization of the active LSR conformation upon occupation of the binding site by the ligand. Alternatively, it is possible that FFA bound to LSR became trapped after binding of the lipoprotein and could no longer be removed by the albumin.

(C) Inhibitory Effect of Lactoferrin on Rat Hepatocyte Membrane LSR. Lactoferrin has previously been shown to delay CMR removal when administered directly into the circulation of the rat (Huettinger et al., 1988). We thus examined whether lactoferrin inhibits oleate-induced binding of [^{125}I]LDL to isolated plasma membranes, and if such an effect was observed, whether it resulted, as with albumin, from the removal of FFA. Figure 9 (panel A) shows that lactoferrin caused the complete inhibition of [^{125}I]LDL binding. However, parallel studies using [^{3}H]oleate (panel B) showed that lactoferrin did not significantly reduce the amount of membrane-associated oleate. This mechanism is different from the inhibitory effect of albumin (right bar, panel A) that was achieved by virtually complete removal of oleate (right bar, panel B).

We next tested whether the exposure to lactoferrin of membranes not preincubated with oleate led to a similar effect. To test this, one set of membranes was incubated first with oleate, then with lactoferrin, and finally with [^{125}I]-

[3] LDL binding to rat plasma membranes preincubated without oleate, which represents less than 10% of LSR activity, is considered to be nonspecific because it is nonsaturable and remains unaffected by protease treatment. Reconstitution experiments (such as that described in Figure 2) showed that the relative importance of this nonspecific binding decreases with the amount of phospholipid (data not shown).

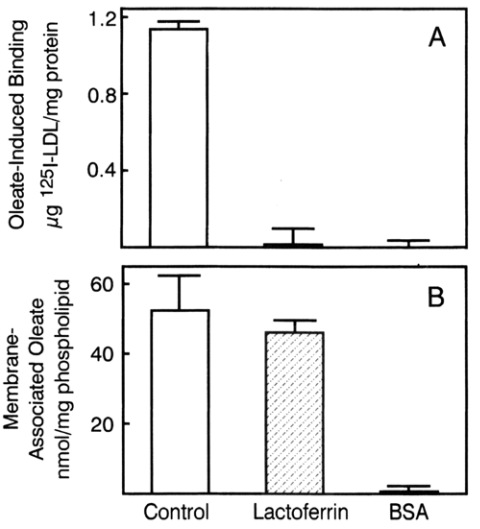

FIGURE 9: Comparison of the effects of lactoferrin and BSA on [^{125}I]LDL binding to and oleate removal from plasma membranes. (A) Three sets of membrane aliquots (100 μg of protein) were incubated at 37 °C for 30 min in the presence of 800 μM oleate and then washed six times with buffer A. Each set was further incubated at 37 °C for 60 min with either buffer A (left bar), with the same buffer supplemented with 2 mg/mL lactoferrin (center bar), or with 1% (w/v) BSA (right bar). After this, the membranes were washed once with buffer A and incubated at 37 °C for 60 min with 10 μg/mL [^{125}I]LDL (specific activity, 170 cpm/ng), and the [^{125}I]LDL bound was measured. Oleate-induced [^{125}I]LDL binding was calculated as described for Figure 2. (B) In parallel with (A), three additional sets of aliquots were incubated at 37 °C for 30 min in the presence of 800 μM [^{3}H]oleate and then washed with buffer A. After incubation exactly as before with buffer A (left bar), lactoferrin (middle bar), or BSA (right bar), these sets were washed once with buffer A, and the amount of [^{3}H]oleate associated with the membranes was determined by using a β-scintillation counter (Beckman LS 6800). Membrane-associated oleate was then calculated from the [^{3}H]oleate specific activity. Each bar represents the mean + SD of triplicate determinations.

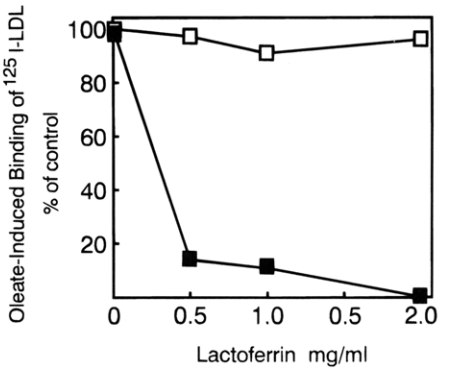

FIGURE 10: Effect of lactoferrin on the active and inactive forms of LSR. One set of membrane aliquots (100 μg of protein) was incubated at 37 °C for 30 min with 800 μM oleate, washed with buffer A, and then incubated at 37 °C for 60 min with the indicated concentrations of lactoferrin (■). A second set of aliquots was conversely treated with the lactoferrin incubation preceding the oleate activation step (□). In both cases, membranes were washed three times after exposure to oleate and once after lactoferrin. All membrane aliquots were then further incubated at 37 °C for 60 min with 10 μg/mL [^{125}I]LDL (specific activity, 170 cpm/ng), and bound [^{125}I]LDL was measured. Values for oleate-induced [^{125}I]-LDL binding were calculated as described for Figure 2 and are expressed as the percent of those obtained in the absence of lactoferrin (■, 2.3 μg/mg; □, 2.4 μg/mg). Each point represents the mean of duplicate determinations.

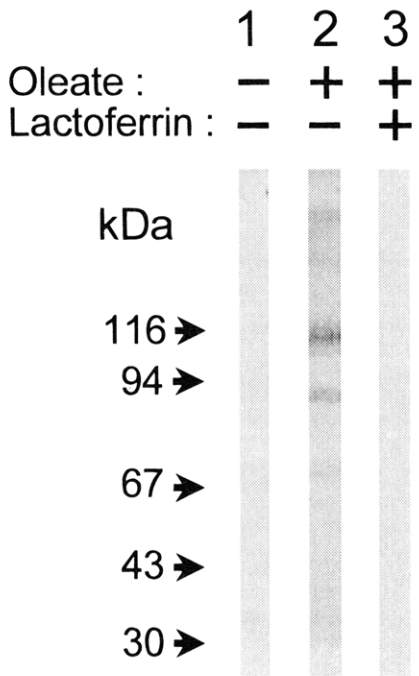

FIGURE 11: [^{125}I]LDL binding to solubilized membrane proteins in the absence or presence of oleate after SDS−PAGE and electrotransfer to nitrocellulose. Proteins were solubilized from rat liver membranes using 13 mM CHAPS, and LSR was partially purified by anion exchange chromatography. The proteins of this LSR-enriched fraction were separated on a 4−12% SDS−polyacrylamide gradient gel and transferred to nitrocellulose. The nitrocellulose strips were blocked with PBS containing 3% (w/v) BSA, washed with PBS, and then incubated at 37 °C for 30 min in the absence of oleate (lane 1) or in the presence of 800 μM oleate (lanes 2 and 3) in buffer A. After they were washed with buffer A, the strips were incubated at 37 °C for 30 min in buffer A (lanes 1 and 2) or in the same buffer supplemented with 5 mg/mL lactoferrin (lane 3). After further washing, the strips were incubated at 37 °C for 60 min with 20 μg/mL [^{125}I]LDL (specific activity, 182 cpm/ng), washed with PBS containing 0.5% (v/v) Triton-X100, and exposed to a phosphor screen. The image of [^{125}I]LDL bound to the nitrocellulose was analyzed by using a PhosphorImager (PhosphorImager SF, Molecular Dynamics).

LDL (Figure 10). A second set of membranes was incubated first with lactoferrin, then with oleate, and finally with [^{125}I]-LDL. In keeping with the data of Figure 9, incubation of oleate-activated LSR with lactoferrin inhibited, in a dose-dependent manner, the binding of [^{125}I]LDL. In contrast, exposure of membranes to lactoferrin prior to the oleate activation step was unable to induce any inhibitory effect. The simplest interpretation of these data is that lactoferrin binds to LSR only when the conformation of the receptor has been modified by FFA to allow ligand recognition. These data thus were consistent with the LSR existing in at least two different conformations, one of these allowing LSR interaction with lactoferrin, LDL, VLDL, and chylomicrons.

Identification of the LSR Protein in Rat Hepatocyte Membranes. To identify the proteins responsible for LSR activity, ligand blotting experiments were performed. A fraction of solubilized membrane protein enriched in LSR by anion exchange chromatography was separated by SDS−PAGE and transferred to nitrocellulose. This procedure revealed two main protein bands of apparent molecular masses under nonreduced conditions of 115 and 90 kDa (Figure 11). A third band of lower intensity was also identified; its apparent molecular mass of 230 kDa was consistent with it representing a dimer of the 115 kDa protein. However, this third band might be a combination of the two other bands. These three proteins appeared to be good candidates for LSR: all bound [^{125}I]LDL after preincubation with oleate, and the binding of all three was inhibited by lactoferrin (lane 3). Results of these experiments also showed that the LSR could be activated by oleate even when the receptor was separated from its phospholipid environment and from other membrane proteins. This is consistent with LSR activation resulting from a conformational shift caused by the direct interaction of FFA with the LSR proteins.

DISCUSSION

The data presented here show that FFA directly induces protein expressed on the external surface of rat hepatocyte plasma membranes to bind lipoprotein. The greater affinity of this LSR for lipoproteins rich in triglycerides provides a mechanism to explain the strong inverse correlation observed between postprandial plasma triglyceride concentration and the apparent number of LSR in the liver.

One of the objectives of this study was to resolve the mechanism by which FFA reveal LSR's binding properties. Biochemical analyses indicated that direct interaction of FFA with the protein leads to a conformational shift that unmasks the lipoprotein binding site. This apparent conformational change remains fully reversible if the ligand binding site is unoccupied. Transition of the protein to its active form was observed with intact membranes, solubilized membrane proteins reconstituted into artificial membranes, and the LSR isolated from membrane lipids, separated from other proteins by SDS−PAGE, and transferred to nitrocellulose. The latter of these observations is consistent with the LSR activation resulting from direct interaction of FFA with the LSR protein itself, not through a change in the conformation of the protein secondary to alteration of the surrounding phospholipid environment. Comparison of the effect of different FFA alone, or in combination, indicated that the same binding site is revealed by the different FFA. However, the length and degree of saturation of the FFA monomeric carbon chain are critical in determining the potency of the FFA to activate LSR. This suggests that the activating effect is limited by the ability of FFA to interact with complementary hydrophobic domains on the protein. It is our hypothesis that filling of these putative domains causes a rearrangement of protein folding and thereby unmasks a series of charged residues capable of binding the ligand, i.e., apo B, apo E, or lactoferrin. Further studies are needed to ascertain whether the FFA interacts directly with the ligand binding site or exerts its effect from another domain of the protein.

An important issue addressed in the present study was whether LSR identified in rat hepatocyte plasma membranes corresponds to the LSR previously identified in intact human FH fibroblasts and primary cultures of rat hepatocytes (Bihain & Yen, 1992; Yen et al., 1994). Four lines of evidence support this concept: (1) hepatocyte plasma membrane LSR is strictly dependent on FFA for its lipoprotein binding capacity; (2) its affinity is greatest for triglyceride-rich lipoproteins; (3) it is Ca^{2+} independent; and (4) binding of the lipoprotein ligand to LSR is lactoferrin sensitive. Furthermore, the apparent molecular masses of the 115 and 90 kDa proteins revealed by ligand blotting using solubilized rat liver proteins are very similar to those of 115 and 85 kDa observed in human FH cell lysate (Yen et al., 1994).

Current data indicate that LSR is expressed in different species and by different tissues. However, it remains unclear why a putative receptor for clearance of triglyceride-rich lipoproteins in the liver is also expressed by FH fibroblasts. One possible explanation is that expression of the receptor in the cultured FH cell line is not a characteristic of fibroblasts *in vivo*. Alternatively, the LSR may serve other, as yet unknown functions in other tissues. LSR tissue distribution is currently under investigation.

The two proteins of 115 and 85 kDa previously identified in FH fibroblasts had suggested that LSR might be composed of two nonidentical subunits. In the current study, ligand blot analysis of rat liver LSR revealed two proteins with similar apparent molecular masses (115 and 90 kDa), as well as a third faint band corresponding to a large protein with an apparent molecular mass of 230 kDa. Two lines of evidence are consistent with all three bands deriving from the same protein: first, all three bind [^{125}I]LDL only after preincubation with FFA, and second, the lipoprotein binding capacity of all three is inhibited by lactoferrin. At this stage, one can only speculate that the 115 kDa band represents the main protein, which can dimerize to produce a 230 kDa protein. The smaller 90 kDa band, which in different preparations varies in intensity relative to the 115 kDa band, may be derived from the latter as a posttranslational modification or as a product of degradation. Alternatively, the 90 kDa band might correspond to an incomplete fragment of a larger multimeric protein; such a fragment would, however, have to retain the FFA and ligand binding domain(s).

Our current hypothesis is that the FFA generated by rapid hydrolysis of chylomicrons serve as signals to activate the LSR. A potential weakness with the present observations is that high concentrations of FFA were required in our assays to maximally recruit the LSR. While fatty acids are present in human plasma at such concentrations, they are predominantly bound to albumin and thus are unlikely to serve as LSR activators. An important technical challenge is to determine whether the *in vivo* local concentration of lipolytic products in the environment bathing the receptor exceeds that in plasma. Also, it is possible that targeting of FFA toward the LSR is more efficient *in vivo* than that observed *in vitro*. Indeed, experiments in which solubilized membrane proteins were reconstituted into artificial membranes indicate that the phospholipid bilayer of intact plasma membranes sequesters much of the FFA and thereby prevents LSR activation. It is possible that such a buffering effect is minimized *in vivo*. This could be envisioned if the LSR is located near the site of FFA production, that is, near hepatic lipase. With LSR and lipase in close proximity, the albumin present at high concentration in plasma also might not be able to achieve *in vivo* the inhibitory effect observed *in vitro*. Consistent with this hypothesis, hepatic lipase has been shown to be expressed on the surface of hepatocytes (Marteau et al., 1988).

At this stage, the physiological function of LSR remains to be established. In view of the mechanism of LSR activation and its ligand specificity, it is tempting to speculate that it serves as a receptor for the cellular uptake of triglyceride-rich lipoproteins undergoing rapid lipolysis. The lack of correlation between the apparent number of hepatocyte LSR and fasting lipid levels does not exclude the possibility that VLDL and LDL, which bind to LSR *in vitro*, are ligands for LSR *in vivo*. However, it suggests that, for the removal of these particles, LSR does not represent the rate-limiting step. In contrast, the inverse correlation between the apparent number of LSR and plasma triglyceride concentration in the fed state is consistent with the number of LSR being rate limiting for the removal of intestinally derived triglyceride-rich lipoproteins. This finding is in agreement with our recent observation that LSR is inhibited by RAP at the relatively high concentrations needed to inhibit CMR clearance *in vivo* (Troussard et al., 1995).

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