The Estradiol-stimulated Lipoprotein Receptor of Rat Liver

A BINDING SITE THAT MEDIATES THE UPTAKE OF RAT LIPOPROTEINS CONTAINING APOPROTEINS B AND E*

(Received for publication, June 17, 1980, and in revised form, July 18, 1980)

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Hepatic catabolism of lipoproteins containing apolipoproteins B or E is enhanced in rats treated with pharmacologic doses of 17α-ethinyl estradiol. Liver membranes prepared from these rats exhibit an increased number of receptor sites that bind 125I-labeled human low density lipoproteins (LDL) in vitro. In the present studies, this estradiol-stimulated hepatic receptor was shown to recognize the following rat lipoproteins: LDL, very low density lipoproteins obtained from liver perfusates (hepatic VLDL), and VLDL-remnants prepared by intravenous injection of hepatic VLDL into functionally eviscerated rats. The receptor also recognized synthetic lamellar complexes of lecithin and rat apoprotein E as well as canine high density lipoproteins containing apoprotein E (apo E-HDL). It did not recognize human HDL or rat HDL deficient in apoprotein E. Much smaller amounts of this high affinity binding site were also found on liver membranes from untreated rats, the number of such sites increasing more than 10-fold after the animals were treated with estradiol. Each of the rat lipoproteins recognized by this receptor was taken up more rapidly by perfused livers from estrogen-treated rats. In addition, enrichment of hepatic VLDL with C-apolipoproteins lowered the ability of these lipoproteins to bind to the estradiol-stimulated receptor and diminished their rate of uptake by the perfused liver of estrogen-treated rats, just as it did in normal rats. The current data indicate that under the influence of pharmacologic doses of estradiol the liver of the rat contains increased amounts of a functional lipoprotein receptor that binds lipoproteins containing apoproteins B and E. This hepatic lipoprotein receptor appears to mediate the uptake and degradation of lipoproteins by the normal liver as well as the liver of estradiol-treated rats. The hepatic receptor bears a close functional resemblance to the LDL receptor previously characterized on extrahepatic cells.

Administration of pharmacologic doses of 17α-ethinyl estradiol to rats elicits an increase in the number of high affinity lipoprotein receptors on liver cell membranes (1). These receptors bind 125I-labeled human low density lipoprotein in vitro. The increased number of LDL binding sites is accompanied by a marked increase in the rate with which the isolated perfused rat liver takes up and degrades human 125I-LDL (2). Several lines of evidence indicate that the lipoprotein binding site that is demonstrable in vitro is responsible for the enhanced LDL uptake in the perfused liver (1, 2). For example, lysine- or arginine-modified LDL preparations, which lose their ability to bind to the receptor in vitro (1), show a greatly reduced uptake by perfused livers of estradiol-treated rats (2).

The hepatic lipoprotein binding site that was induced by estradiol resembles in several respects the LDL receptor previously studied in human fibroblasts (3). In addition to its failure to bind modified LDL preparations, the estradiol-stimulated receptor is inhibited by EDTA and destroyed by pronase (1). Moreover, the estradiol-stimulated hepatic receptor, like the fibroblast LDL receptor, fails to bind human high density lipoproteins (1). The LDL receptor of human fibroblasts is known to bind lipoproteins that contain apoprotein E, such as certain types of very low density lipoproteins, as well as LDL, which contains predominantly apoprotein B (3, 4). Similarly, perfused livers from estrogen-treated rats exhibit accelerated rates of uptake and catabolism of rat HDL that contains apoprotein E, but not human or rat HDL that are deficient in this protein (2).

In the present experiments, we have explored the specificity of the estradiol-stimulated binding site in rat liver by studying its ability to bind homologous lipoproteins that contain apoprotein B, apoprotein E, or both. For this purpose, we have used radiolabeled VLDL from liver perfusates, before and after enrichment with C apolipoproteins, VLDL-remnants, LDL, and isolated apoprotein E, which has been incorporated into complexes with phospholipid. In each case, we have measured the effect of treatment of rats with estradiol upon the binding of the lipoprotein to rat liver membranes and the uptake of the lipoprotein by the perfused liver. The binding affinity and hepatic uptake were higher for lipoprotein particles containing apoprotein E than for particles containing predominantly or solely apoprotein B. Treatment of rats with estradiol had little effect on apparent binding affinity, but stimulated binding capacity for each of these lipoproteins more than 10-fold.

* This research was supported by grants from the United States Public Health Service (HL-20948, HL-14237, and HL-06285). Paul Harvill and Jeffrey Weissman provided excellent technical assistance.

1 The abbreviations used are: LDL, low density lipoproteins; VLDL, very low density lipoproteins; apo, apoprotein; HDL, high density lipoproteins; apo E-HDL, a lipoprotein appearing in the plasma of cholesterol-fed dogs and consisting predominantly of apoprotein E; SDS, sodium dodecyl sulfate.
Estradiol-stimulated Lipoprotein Receptor

EXPERIMENTAL PROCEDURES

Materials—[125I]cholesterol ester (Cat No. 644-14, Pharmacia, Piscataway, NJ), [125I]triglycerides (Cat. No. 1927-02, and bovine serum albumin (Cat. No. 8A-130) were obtained from Sigma Chemical Co. Sodium [125I]cholesterol (500 mcg/ml) was obtained from Amersham/International (I-355-0500 Clammy). [125I]cholesterol (500 mcg/ml) was obtained from Amersham/International (I-344-0500 Clammy). Lipids were isolated from rabbit liver by the method of Lary et al. (12, 13). All reagents were obtained from Steraloids Laboratories. Other materials were obtained from previously reported sources (14, 15).

Animals and Urine—Male Sprague-Dawley rats, weighing 300–350 g and maintained on a diet containing 5% cholesterol, 15% fat, and tap water, were used for preparation of liver membranes, as described previously (16). Animals were fed in the presence of cholesterol and estradiol, and included a check or money order for $1.20 per set of Figs. 1–11.

Preparation of lipoprotein—Rat LDL (5 mg I-251/mL), rat HDL (0.1 mg I-251/mL), and bovine LDL (0.5 mg I-251/mL) was prepared by precipitation with polyethylene glycol as described previously (12, 13, 17). Rat hepatic VLDL were isolated from rat LDL (obtained from Rat LDL, Inc., and cholesterolized with 30 mg/dl) were used for specific binding measurements for 125I labeling with (5) and without (3) specified concentration of rat LDL. Rat hepatic VLDL were isolated from the cholesterolized lipoprotein fraction (6) and were used for specific binding measurements for (3) and (5).

Binding of [125I]cholesterol to rat liver membranes—Analytical binding studies were performed under standard conditions. Changes in the amount of bound lipoprotein were measured by standard procedures, as previously described (10, 11). The apparent affinity of the membrane to bind lipoprotein was measured by a simple method (14, 15). The amount of labeled lipoprotein bound to liver membranes was determined by a simple method (14, 15). The amount of labeled lipoprotein bound to liver membranes was determined by a simple method (14, 15).

RESULTS

Binding of 125I-labeled Rat Lipoproteins to Liver Membranes—Fig. 1, A and B shows saturation curves for the binding of rat 125I-LDL to liver membranes prepared from control and estradiol-treated rats. As a control for nonspecific binding, we performed the incubations in the absence and presence of EDTA which is known to abolish the specific binding of human 125I-LDL to the estradiol-induced hepatic lipoprotein receptor (1). In the presence of 10 mM EDTA, the amount of rat 125I-LDL bound increased in a linear fashion with increasing concentrations of 125I-LDL. As shown in Fig. 1C, the slope of this line was similar in the control and estradiol-treated rats. In other experiments, we have shown that this EDTA-resistant binding is not reduced in the presence of an excess of unlabeled LDL. Presumably it represents nonspecific binding or trapping of the 125I-LDL by the membranes. In the absence of EDTA, the binding curves were nonlinear, suggesting the presence of saturable, high affinity binding sites that were abolished when EDTA was present. In contrast to the control rats, membranes from estradiol-treated animals showed evidence for a single high affinity binding site with a calculated Kd of 3.4 μg of protein/ml. The maximal binding at saturation was equivalent to about 2200 ng of LDL protein bound/mg of membrane protein. As expected from the binding curves, membranes from control rats showed much less high affinity binding than the membranes from the estradiol-treated rats as compared with the controls.

The estradiol-mediated increase in the high affinity binding of rat 125I-LDL was also apparent when the specific binding data were plotted according to the method of Scatchard (16). As shown in Fig. 1C, the slope of this line was similar in the control and estradiol-treated rats. In other experiments, we have shown that this EDTA-resistant binding or trapping of the 125I-LDL by the membranes. In the absence of EDTA, the binding curves were nonlinear, suggesting the presence of saturable, high affinity binding sites that were abolished when EDTA was present. In contrast to the control rats, membranes from estradiol-treated animals showed evidence for a single high affinity binding site with a calculated Kd of 3.4 μg of protein/ml. The maximal binding at saturation was equivalent to about 2200 ng of LDL protein bound/mg of membrane protein. As expected from the binding curves, membranes from control rats showed much less high affinity binding when plotted by the Scatchard method on the same scale as the estradiol-treated membranes (Fig. 1C).

When the data from the control rats were plotted on an expanded scale (Fig. 2A), the nonlinear binding curve could be resolved into two components by the graphical method of Rosenthal (11). The apparent Kd for the highest affinity component (2.0 μg of protein/ml) was similar to that of the low affinity component (2.0 μg of protein/ml) divided by the concentration of unbound lipoprotein in the reaction mixture (μg of protein/ml).

2 Portions of this paper (including "Experimental Procedures," and Figs. 3, 4, 5, and 6) are presented in miniprint. Miniprint is easier to read with the aid of a standard magnifying glass. Full size photographs are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 80M-1220, cite author(s), and include a check or money order for $1.20 per set of photographs. Full size photographs are also included in the microfilm edition of the Journal that is available from Waverly Press.
control animals was only \( \frac{1}{2} \) as great as in the treated animals (70 versus 2200 ng of LDL-protein bound/mg of membrane protein) (compare Fig. 2A and Fig. 1C).

The liver membranes from control and estradiol-treated rats also bound hepatic \( ^{125}\text{I}-\text{VLDL} \) isolated from perfusates of rat livers (Fig. 3). In membranes from both the control and estradiol-treated animals, the binding of the hepatic \( ^{125}\text{I}-\text{VLDL} \) was competitively reduced in the presence of an excess of unlabeled hepatic VLDL. At concentrations of hepatic \( ^{125}\text{I}-\text{VLDL} \) below 2 \( \mu \)g of protein/ml, the amount of specific or displaceable binding was more than 10-fold higher in the membranes from the estradiol-treated rats as compared with the control rats. A Scatchard plot of these data further illustrated the difference in the number of high affinity binding sites (Fig. 3C). The calculated \( K_d \) for binding in the estradiol-treated rats was about 0.3 \( \mu \)g of protein/ml, which was similar to the high affinity component seen with membranes from estradiol-treated livers. However, as was the case with rat \( ^{125}\text{I}-\text{LDL} \), the number of high affinity binding sites for hepatic \( ^{125}\text{I}-\text{VLDL} \) in the control rats was only \( \frac{1}{2} \) as great as in the estradiol-treated animals (36 versus 1100 ng of protein bound/mg of membrane protein) (compare Fig. 2B and Fig. 3C).

The binding of hepatic \( ^{125}\text{I}-\text{VLDL} \) to rat liver membranes was inhibited by 95% by the inclusion of 10 mM EDTA in the incubation medium (data not shown). The high affinity binding at 0°C reached equilibrium by 60 min at an \( ^{125}\text{I}-\text{VLDL} \) concentration of 0.13 \( \mu \)g of protein/ml and by 30 min at an \( ^{125}\text{I}-\text{VLDL} \) concentration of 4.5 \( \mu \)g of protein/ml. Specific binding of hepatic \( ^{125}\text{I}-\text{VLDL} \) was 2- to 3-fold higher at 4°C than at 37°C in liver membranes from either control or estradiol-treated rats. No specific binding of \( ^{125}\text{I}-\text{VLDL} \) could be detected when the lipoprotein was incubated with membranes prepared from erythrocytes of control or estradiol-treated rats.

The entire hepatic \( ^{125}\text{I}-\text{VLDL} \) particle appeared to bind to the hepatic receptor. Thus, in a typical preparation of hepatic \( ^{125}\text{I}-\text{VLDL} \) about 20% of the radioactive iodine was extractable into organic solvents, presumably because it was attached to lipid molecules. The same proportion of radioactivity was extractable from the membranes that had bound hepatic \( ^{125}\text{I}-\text{VLDL} \), i.e., the membranes appeared to be binding both the lipid and the protein portions of the hepatic VLDL in equivalent amounts. The various protein components of VLDL were also bound roughly in proportion to their occurrence in the VLDL particle. SDS-polyacrylamide gel electrophoresis of the hepatic \( ^{125}\text{I}-\text{VLDL} \) followed by autoradiography using previously described methods (16) revealed labeled bands that corresponded to B, E, and C apoproteins. When liver membranes were allowed to bind this hepatic \( ^{125}\text{I}-\text{VLDL} \), and were then isolated by centrifugation, solubilized with SDS, and subjected to electrophoresis, the distribution of labeled bands was similar to that of the hepatic \( ^{125}\text{I}-\text{VLDL} \) preparation. Binding of all of the protein components was inhibited in parallel by the inclusion of an excess of unlabeled hepatic VLDL in the incubations.

Incubation of rat hepatic \( ^{125}\text{I}-\text{VLDL} \) with VLDL-free rat plasma \( (d > 1.019 \text{g/ml fraction}) \) has been shown previously to result in the transfer of C apoproteins to the \( ^{125}\text{I}-\text{VLDL} \) with a concomitant decrease in the rate at which the \( ^{125}\text{I}-\text{VLDL} \) is taken up by the perfused rat liver (5). Fig. 4 shows that incubation of the \( ^{125}\text{I}-\text{VLDL} \) with VLDL-free plasma resulted in a decreased binding of the lipoprotein to liver.
membranes from estrogen-treated rats. Similar findings were obtained with liver membranes from control rats.

1-VLDL-remnants prepared by injection of hepatic $^{125}$I-VLDL into functionally hepatectomized rats also bound to the estradiol-stimulated receptor of rat liver membranes (Fig. 5B). The binding was competed by an excess of unlabeled VLDL-remnants. By Scatchard plot analysis (Fig. 5C), the apparent $K_d$ for the high affinity binding of $^{125}$I-VLDL-remnants to the membranes from estradiol-treated animals was about 0.9 µg of protein/ml. Analysis of the data from the control animals using expanded Scatchard plots (Fig. 2C) revealed the existence of a high affinity component that had an apparent $K_d$ similar to that of the estradiol-treated membranes (0.4 µg of protein/ml). The maximal number of high affinity sites was much lower in control membranes as compared with those of the estradiol-treated animals (maximal binding of 40 versus 700 ng of protein bound/mg of membrane protein) (compare Fig. 2C and Fig. 5C).

The major protein components of the rat $^{125}$I-VLDL used in these studies were B, E, and C apoproteins. On the other hand, rat (7, 17) as well as human LDL contain apoprotein B as the predominant apoprotein component. To determine whether a lipoprotein containing solely the E apoprotein could bind to the hepatic lipoprotein receptor of estradiol-treated rats, we isolated rat apo E by gel filtration and incorporated the protein into phospholipid complexes (6). Fig. 6 shows that liver membranes from control animals bound these $^{125}$I-apo E-lecithin complexes in a saturable reaction in which the total binding was reduced in the presence of EDTA. Membranes from estradiol-treated animals showed an increase in binding of the $^{125}$I-apo E-lecithin complexes that was most marked at low concentrations of the lipoprotein. The difference between the control and estradiol-treated membranes was also apparent in Scatchard plots (Fig. 6C). On an expanded scale the control membranes showed evidence for a high affinity site with an apparent $K_d$ of 0.15 µg of protein/ml (Fig. 2D). The maximal amount of binding to this site was equal to about 20 ng of protein/mg of membrane protein (Fig. 2D). In the estradiol-treated animals, there was a 20-fold increase in the number of these high affinity binding sites (Fig. 6C). The apparent $K_d$ for the high affinity site was similar to that of the controls (0.59 µg of protein/ml).

The above data indicate that liver membranes from both control and estradiol-treated rats have high affinity binding sites that recognize several rat lipoproteins, including rat $^{125}$I-LDL, rat hepatic $^{125}$I-VLDL, and rat $^{125}$I-VLDL-remnants. The number of these sites is greatly increased in liver membranes from estrogen-treated rats, which also recognize human $^{125}$I-LDL (1). To confirm that these lipoproteins were binding to the same receptor site, we performed a series of competition experiments (Fig. 7). Human LDL and rat hepatic VLDL both competed with human $^{125}$I-LDL for binding to the high affinity receptor of the estradiol-treated membranes, whereas human HDL (d 1.20 to 1.215 g/ml) was much less effective (Fig. 7A). Similarly, when rat hepatic $^{125}$I-VLDL was used as the labeled ligand, human LDL competed, as did unlabeled rat hepatic VLDL, whereas human HDL did not effectively compete (Fig. 7B). Figure 7C shows an experiment in which the binding of rat hepatic $^{125}$I-VLDL was competed by unlabeled rat hepatic

3 EDTA at 10 mM reduced the high affinity binding of rat $^{125}$I-apo E-lecithin complexes to liver membranes from estradiol-treated rats by about 60%. To achieve complete inhibition of this binding, EDTA concentrations of 60 mM were required. These findings are in contrast to the observations with human $^{125}$I-LDL, rat $^{125}$I-LDL, rat hepatic $^{125}$I-VLDL, and rat $^{125}$I-VLDL-remnants in which 10 mM EDTA produces a complete inhibition of high affinity binding to liver membranes.

VLDL and also by rat VLDL-remnants and rat LDL. On the other hand, rat HDL (d 1.075 to 1.215 g/ml) was much less effective in competing for this binding. Thus, human and rat LDL, rat hepatic VLDL, and rat VLDL-remnants all appeared capable of binding to the same receptor site, whereas human and rat HDL were much less effective.

Uptake and Degradation of Rat VLDL Preparations by Perfused Livers —Fig. 8 shows an experiment in which rat hepatic $^{125}$I-VLDL were perfused in a recycling system through the livers of control and estradiol-treated rats. In control livers, 25% of the trichloroacetic acid-precipitable $^{125}$I was recovered in lipoproteins of d < 1.24 g/ml after 2 h, whereas 60% was removed by livers from estradiol-treated rats in the same period. Livers from control rats released 15% of the added $^{125}$I-VLDL as acid-soluble $^{125}$I into the perfusate within 2 h, whereas livers from estradiol-treated rats released 30% (Fig. 8). Of the trichloroacetic acid-precipitable $^{125}$I remaining in the perfusate after 2 h, 40% was recovered in lipoproteins of d < 1.006 g/ml for control livers and 24% for livers from estradiol-treated rats. At the end of
the perfusion period, livers from control rats contained 20% of the added 125I and livers from estradiol-treated rats contained 30%. Thus, these experiments demonstrate that the livers from the estradiol-treated rats took up and degraded rat hepatic 125I-VLDL at a faster rate than the livers from control rats.

To study the uptake of the cholesteryl ester component of rat hepatic VLDL, the fate of the [3H]cholesteryl esters of the hepatic VLDL added to perfusates of rat livers was followed (Fig. 9). The initial rate of removal of [3H]cholesteryl esters of the hepatic VLDL (Fig. 9A) was much greater in livers from estradiol-treated rats than it was in control livers. After 20 min, livers from control rats had taken up about 30% of the added [3H]cholesteryl ester, whereas livers from estradiol-treated rats had taken up about 70%. Thereafter, livers from the estradiol-treated animals apparently resecreted [3H]cholesteryl ester back into the perfusate. It is noteworthy that hepatic VLDL obtained from perfusates of livers from estradiol-treated rats contained much more cholesteryl ester and less triglyceride per particle than did the hepatic VLDL from control livers (Table I).

Figure 9B shows an experiment in which the rat livers were perfused with incubated VLDL (rat hepatic VLDL that had been enriched in C apoproteins by incubation with VLDL-free rat plasma). The [3H]cholesteryl esters of the incubated hepatic VLDL were taken up much more slowly than those from the unincubated hepatic VLDL (compare Fig. 9B with Fig. 9A). Despite the decrease in the rate of uptake of the incubated VLDL, the difference between the control and estradiol-treated animals persisted. That is, after 30 min about 5% of the [3H]cholesteryl esters of incubated VLDL had been taken up by livers from control rats, but about 30% by livers from estradiol-treated rats.

In an additional experiment, rat VLDL-remnants labeled with [3H]cholesterol and [14C]palmitate were isolated from the serum of functionally eviscerated rats 15 min after the injection of double-labeled hepatic VLDL containing 11 to 17 mg of triglycerides. When these VLDL-remnants, containing 0.4 mg of triglycerides, were perfused through recipient livers in a single pass, livers from estradiol-treated rats took up twice as much [3H]cholesteryl esters as livers from control rats (Table II).

DISCUSSION

Table III summarizes the results of the current studies of the binding of 125I-labeled lipoproteins to the high affinity binding sites on liver membranes from control and estradiol-treated rats. The apparent Kd and maximal binding capacity for each lipoprotein were calculated from Scatchard plots and represent the mean values obtained from several experiments as indicated. For rat LDL, rat hepatic VLDL, and rat VLDL-remnants, there was evidence for a high affinity binding site in the liver membranes from both the control and estradiol-treated rats. In each case, the apparent number of high affinity binding sites (Bmax) increased by more than 10-fold after estradiol treatment. With respect to each lipoprotein, the apparent affinity of the binding site was similar in the membranes from control and estradiol-treated rats. On a molar basis, rat hepatic VLDL and VLDL-remnants showed the highest affinities, but the lowest number of binding sites. The affinity (M x 10^-9) for rat LDL was about 10-fold lower than that for the two rat VLDL preparations, but the number of binding sites for rat LDL was about 5-fold greater. These results with liver membranes are similar to the corresponding values obtained by Innerarity et al. from studies of the binding of rat LDL and VLDL to intact rat fibroblasts (17). Both systems bind VLDL with a higher affinity and a lower capacity than LDL.

Phospholipid complexes of rat apoprotein E also bound
Rats had been incubated with the serum of hepatic VLDL (radiolabeled with [3H]cholesteryl esters and containing 5 to 10 mg of triglycerides) and the incubated VLDL (radiolabeled with [3H]cholesteryl esters and containing 4 to 15 mg of triglycerides) were added to each perfusate in a recycling system as described under “Experimental Procedures.” The nonincubated hepatic VLDL contained 140 to 174 μg of protein/mg of triglycerides and the incubated VLDL contained 224 to 267 μg of protein/mg of triglycerides.

**Table I**

| Source of hepatic VLDL | Cholesteryl esters | Triglycerides | Free cholesterol | Phospholipids | Total protein | Apo E | Apo A-I |
|------------------------|-------------------|---------------|------------------|---------------|--------------|-------|---------|
| Control rats (n = 5)   | 1.5 ± 0.89        | 80.8 ± 3.4    | 3.1 ± 1.0        | 14.6 ± 3.5    | 118 ± 50     | 17 ± 5 | 2 ± 1   |
| Estradiol-treated rats (n = 3) | 16.6 ± 4.4         | 21.1 ± 4.2    | 4.0 ± 0.5        | 17.2 ± 1.1    | 90 ± 17      | 13 ± 6 | 7 ± 1   |

* Values from Ref. 5. VLDL from control and estradiol-treated rats were isolated from perfusates after 2 h of a recycling perfusion.

**Table II**

**Uptake of [3H]cholesteryl esters of rat VLDL-remnants by perfused livers from control and estradiol-treated rats**

The uptake of rat VLDL-remnants (~0.4 mg of triglycerides) radiolabeled with [3H]cholesteryl esters by perfused rat livers was measured by the single pass technique as described under “Experimental Procedures.”

| Measured quantity | Control (n = 5) | Estradiol-treated (n = 5) |
|-------------------|----------------|-------------------------|
| [3H]Cholesteryl esters recovered in perfusates | 82.6 ± 6.3% | 64.8 ± 13.1 | p < 0.05 |
| [3H]Cholesteryl esters recovered in livers | 18.4 ± 9.2% | 34.4 ± 10.2 | p < 0.05 |
| Total recovery of [3H]-cholesterol esters | 101.1 ± 8.5% | 99.2 ± 6.1 |

* Mean ± S.D.

A. Protein component of the three rat LDL preparations, as judged by SDS-polyacrylamide gel electrophoresis or radioimmunoassay (see legend to Table III). This is equivalent to less than 1 molecule of apoprotein E in each LDL particle.

On the basis of current data, it appears that human LDL, rat LDL, rat hepatic VLDL, and rat VLDL-remnants bind to the same receptor in the liver membranes from estradiol-treated rats. This conclusion is supported by several lines of evidence. First, the apparent number of binding sites for each of these lipoproteins increased markedly after estradiol treatment. Second, each of these lipoproteins showed cross-competition when studied in competitive binding assays of the type shown in Fig. 7. Third, the high affinity binding of each of these lipoproteins was completely inhibited by 10 mM EDTA, and was also abolished by treatment of the liver membranes with 5 μg/ml of promace (data not shown). It seems likely that the rat 125I-apo E-lecithin complexes and the canine 125I-apo E-HDL are recognized by the same estradiol-stimulated receptor that binds rat VLDL and rat and human LDL. Indeed, unlabeled canine apo E-HDL at 2.5 μg of protein/ml completely inhibited the high affinity binding of 1 μg of protein/ml of rat hepatic 125I-VLDL (data not shown).

Although each of the above lipoproteins appeared able to bind to the same receptor site, the number of particles bound at saturation was markedly different for the different lipoproteins (Table III). These differences may have been due to the ability of some lipoproteins, such as VLDL and the apo E-enriched lipoproteins, to bind to multiple receptors, thereby achieving a higher affinity but a lower maximal binding capacity than LDL. Evidence from Pitas et al. indicates that such a mechanism occurs in cultured human fibroblasts (19). These cells exhibit a higher affinity and lower capacity for binding apo E-HDL, as compared with LDL, apparently because a single apo E-HDL particle binds to four receptor sites (19).
Liver membranes were prepared from rats that were treated with propylene glycol (control) or with 17α-ethyl estradiol for 7 days. The binding reactions for the 125I-labeled lipoprotein fractions were carried out as described under “Experimental Procedures.” Scatchard plots of the binding data were constructed, and the amount of lipoproteins bound at receptor saturation (Bmax) and the equilibrium dissociation constants (Kd) of the high affinity site (K1) were calculated as described under “Experimental Procedures.” Each value represents the average of the indicated number of experiments. The molar concentration of each lipoprotein particle was calculated from the indicated molecular weight and protein mass.

### Table III: Characteristics of the binding of various lipoproteins to liver membranes from control and estradiol-treated rats

| Lipoproteins                          | Estimated molecular weight of particle | Protein content of particle | Source of liver membranes | Equilibrium dissociation constant (Kd) | Amount of lipoprotein bound at saturation (Bmax) |
|---------------------------------------|---------------------------------------|-----------------------------|---------------------------|---------------------------------------|-------------------------------------------------|
| Rat LDL                               | 3 × 10^6                               | % mass                      | Control                   | 1.2 × 10^-6                            | 0.50 × 10^-12 µg protein/mg membrane protein     |
| Rat hepatic VLDL                      | 40                                     | 9                           | Estradiol (3)             | 3.0 × 10^-6                            | 5.0 × 10^-12 µg protein/mg membrane protein      |
| Rat VLDL-remnants                     | 21                                     | 12                          | Control                   | 0.56 × 10^-6                           | 0.16 × 10^-12 µg protein/mg membrane protein     |
| Rat Apo E-lecithin complexes          | 1                                      | 15                          | Estradiol (2)             | 1.1 × 10^-6                            | 0.15 × 10^-12 µg protein/mg membrane protein     |
| Human LDL                             | 3                                      | 20                          | Control                   | 0.37 × 10^-6                           | 0.81 × 10^-12 µg protein/mg membrane protein     |
|                                      |                                        |                             | Estradiol (4)             | 0.31 × 10^-6                           | 0.57 × 10^-12 µg protein/mg membrane protein     |
|                                      |                                        |                             | Control                   | 0.23 × 10^-6                           | 0.38 × 10^-12 µg protein/mg membrane protein     |

**Notes:**
- These results were obtained with rat LDL that was prepared by two different procedures. One preparation was purified by ultracentrifugation as described under “Experimental Procedures,” and the other was provided by Drs. Robert W. Mahley and Thomas Innerarity. The latter samples were prepared by ultracentrifugation and Geon-Pevikon electrophoresis (4, 17). Apoprotein B comprises greater than 85% of the total protein mass of the rat LDL prepared by the two different procedures, as judged by either radioimmunoassay (7) or polyacrylamide SDS-gel electrophoresis (17).

The hepatic membrane binding site that recognizes VLDL and LDL does not appear to recognize either human HDL3 (d 1.120 to 1.215 g/ml) or rat HDL (d 1.075 to 1.215 g/ml) prepared by ultracentrifugation (Ref. 1 and Fig. 7). The latter lipoprotein loses much of its apoprotein E during isolation (2, 20). In addition to the hepatic receptor site that recognizes lipoproteins containing apoproteins B and E, rat liver membranes express an additional binding site that recognizes human 125I-HDL1 (1). In contrast to the site that binds LDL, VLDL, and apo E-enriched lipoproteins, the HDL binding site does not show competition by LDL. Moreover, the number of these HDL sites in the liver membranes of estradiol-treated rats does not increase (1), as expected from the lack of augmented catabolism in perfused livers from such animals (2). In experiments not shown, we have made similar observations on the binding to liver membranes of rat 125I-HDL that is deficient in apo E.

In addition to the estradiol-stimulated receptor as summarized in Table III, liver membranes from untreated rats expressed at least one other lipoprotein binding site that was of lower affinity, but was nevertheless susceptible to competition with an excess of unlabelled ligand. The observed displaceable or specific binding in the experiments of Figs. 1A, 3A, 5A, and 6A represented the sum of these two binding sites and was difficult to evaluate from saturation curves alone. By the Scatchard method, however, the data yielded nonlinear plots that could be resolved into two lines by the graphical method of Rosenthal (11). The calculated Kd for the highest affinity site was similar to the Kd for the receptor observed in membranes from livers of estrogen-treated rats. This similarity supports the interpretation that the nonlinear Scatchard plots in the control rats are created by the presence of several binding sites of differing affinities. The highest affinity binding site appears to be the same as the high affinity binding site in the membranes from estradiol-treated rats. The lower affinity site was not detected in the membranes from estradiol-treated rats, possibly because the increased number of the high affinity sites made it technically difficult to evaluate a quantitatively minor site with lower affinity. Further studies with purified receptors will be necessary to corroborate the suggestion that the nonlinear Scatchard plots are generated by the presence of multiple binding sites.

The estradiol-mediated increase in high affinity lipoprotein binding sites on rat liver membranes was associated with an increase in the rate at which the intact perfused livers took up and degraded the lipoproteins. Thus, hepatic 125I-VLDL were taken up 2- to 3-fold more rapidly by livers from estradiol-treated rats as compared with controls. The rate of uptake of [1H]cholesterol esters in the core of the lipoprotein was also increased by 2- to 3-fold in the estradiol-treated livers, suggesting that the lipoprotein was being taken up as an intact particle. Finally, incubation of the rat hepatic VLDL with plasma, which reduced the ability of the lipoprotein to bind to the liver membrane binding site (Fig. 4), reduced the rate at which its [1H]-labeled cholesteryl esters were taken up by the perfused liver (Fig. 9). These results suggest that the high affinity lipoprotein binding site demonstrable in vitro mediated the uptake of the lipoproteins in vivo. This conclusion is further supported by the previous demonstration that the uptake of rat 125I-apo E-lecithin complexes is increased by about 10-fold in perfused livers of estradiol-treated rats (6), an increase that correlates with the 25-fold increase in binding sites for rat 125I-apo E-lecithin complexes observed in the current study.
Estradiol-stimulated Lipoprotein Receptor

Considered together with our previous studies (1, 2), the findings reported here indicate that pharmacologic doses of 17α-ethinyl estradiol elicit an increase in the number of high affinity lipoprotein receptors in rat liver. This increase appears to involve a single species of receptor that recognizes human LDL, rat LDL, rat VLDL, canine apo E-HDL, and an artificial lipoprotein containing rat apoprotein E. The increase in these binding sites is very likely responsible for the increased ability of the liver to remove these lipoproteins from the circulation and to degrade them. The same receptor is also present in livers of rats that have not been treated with ethinyl estradiol, albeit in much lower numbers. Further studies will be necessary to determine the extent to which this receptor is responsible for the hepatic uptake of LDL and VLDL in normal rats in vivo.

An important point to emerge from the current studies relates to the remarkable similarities in properties shared by the estradiol-stimulated receptor of rat liver and the LDL receptor of human fibroblasts and other nonhepatic cell types. Both receptor systems show the following similarities: 1) they both recognize lipoproteins containing apoproteins B and E; 2) the affinity for apo E-containing lipoproteins is greater than for apo B-containing lipoproteins; 3) neither receptor recognizes typical HDL; 4) neither receptor recognizes lysine-modified or arginine-modified LDL; 5) the binding reaction in both systems is inhibited by EDTA and restored by calcium; 6) both receptors are sensitive to destruction by pronase; and 7) the initial binding event is coupled to cellular uptake and degradation of the lipoprotein. In addition, autoradiographic studies indicate that 85 to 95% of the initial binding of intravenously injected 125I-LDL is to the plasma membrane of parenchymal cells of livers from both normal and estrogen-treated rats. This binding is rapidly followed by endocytosis and lysosomal catabolism, as with the LDL receptor of nonhepatic cells.

There are several hypotheses that can be advanced to explain these similarities. First, the estradiol-stimulated receptor in liver may be produced by the same gene as the LDL receptor of nonhepatic cells and this receptor may normally function in the hepatic uptake of VLDL-remnants as well as LDL. Estradiol may simply stimulate the liver to produce an increased number of such receptors. A second possibility is that the hepatic receptor that is stimulated by estradiol is the product of a distinct gene that is expressed only in hepatocytes and normally functions in the hepatic uptake of VLDL-remnants. Treatment of rats with estradiol may simply enhance the number of VLDL-remnant receptors. If this is true, then the properties of the hepatic VLDL-remnant receptor are remarkably similar to those of the extrahepatic LDL receptor, suggesting that the two genes specifying these two receptors are similar but not identical. Our present state of knowledge does not allow us to decide between these two possibilities. Further studies using antibodies prepared against purified receptors from the liver and from extrahepatic tissues may be required to answer this question.

REFERENCES
1. Kovanen, P. T., Brown, M. S., and Goldstein, J. L. (1979) J. Biol. Chem. 254, 11367-11373
2. Chao, Y-S., Windler, E. E., Chen, G. C., and Havel, R. J. (1979) J. Biol. Chem. 254, 11360-11366
3. Goldstein, J. L., and Brown, M. S. (1977) Annu. Rev. Biochem. 46, 897-930
4. Mahley, R. W., and Innerarity, T. L. (1978) in Drugs, Lipid Metabolism, and Atherosclerosis pp. 99-127, Plenum Publishing Corporation, New York
5. Windler, E., Chao, Y-S., and Havel, R. J. (1980) J. Biol. Chem. 255, 5475-5480
6. Havel, R. J., Chao, Y-S., Windler, E. E., Kotite, and Guo, L. S. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 4349-4353
7. Sigurdsson, G., Noel, S.-P., and Havel, R. J. (1978) J. Lipid Res. 19, 629-634
8. Havel, R. J., Felts, J., and Van Duyne, C. (1962) J. Lipid Res. 3, 297-308
9. Basu, S. K., Goldstein, J. L., and Brown, M. S. (1978) J. Biol. Chem. 253, 3852-3856
10. Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51, 660-672
11. Rosenthal, H. E. (1967) Anal. Biochem. 20, 525-532
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
13. Faerber, M., Havel, R. J., and Imaizumi, K. (1977) Biochim. Biophys. Acta 490, 144-155
14. Faerber, M., Havel, R. J., and Felker, T. E. (1976) Biochim. Biophys. Acta 444, 56-58
15. Faerber, O., and Havel, R. J. (1975) J. Clin. Invest. 55, 1210-1218
16. Kovanen, P. T., Schneider, W. J., Hillman, G. M., Goldstein, J. L., and Brown, M. S. (1979) J. Biol. Chem. 254, 5498-5506
17. Pitas, R. E., Innerarity, T. L., and Mahley, R. W. (1980) J. Biol. Chem. 255, 5454-5460
18. Innerarity, T. L., Pitas, R. E., and Mahley, R. W. (1979) J. Biol. Chem. 254, 4186-4190
19. Pitas, R. E., Innerarity, T. L., Arnold, K. S., and Mahley, R. W. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 2311-2315
20. Faerber, M., Havel, R. J., and Imaizumi, K. (1977) Biochem. Med. 17, 347-355