A comparison of the low-density-lipoprotein receptor from bovine adrenal cortex, rabbit and rat liver and adrenal glands by lipoprotein blotting

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This paper describes the use of lipoprotein blotting to detect low-density-lipoprotein (LDL) receptors in rat and rabbit liver and adrenal glands and in bovine adrenal glands. Using this technique we show that the rabbit and rat liver LDL receptors have M_r values of 128 000 and 145 000 respectively. M_r values for the rabbit, rat and bovine adrenal receptors are 131 000, 142 000 and 132 000 respectively. Differences between the bovine adrenal and rat liver receptors are not due to differences in the degree of sialylation. Lipoprotein blotting can be used to detect dietary- and drug-induced changes in the concentrations of LDL receptors. When rabbits are fed on a cholesterol-rich diet, liver LDL receptors cannot be detected, consistent with the suppression of hepatic LDL receptors by cholesterol feeding. Pharmacological doses of 17α -ethinyloestradiol cause a marked increase in hepatic LDL-receptor activity in the rat. This is accompanied by a corresponding increase in the number of LDL receptors detected by lipoprotein blotting. The M_r of the induced receptor is identical with that of the receptor from control rats, which suggests that the induced receptors are produced by the same gene as LDL receptors normally present in the liver.

Receptors for plasma LDL exist on the cell surface of most cultured mammalian cells. This receptor recognizes lipoproteins which contain apo B or apo E. After binding to the LDL receptor, LDL is taken into the cell by absorptive endocytosis and is degraded in lysosomes, providing cholesterol for use by the cell (Goldstein & Brown, 1977). Cholesterol supplied in this manner is used for diverse purposes, which include membrane synthesis (Brown & Goldstein, 1979), production of bile acids in the liver (Slater *et al.*, 1980) and production of steroid hormones in the adrenal cortex (Brown *et al.*, 1979).

In vivo, the liver is the major site for the removal of plasma lipoprotein cholesterol. Several lines of evidence show that hepatic LDL receptors play a major role in modulating this process. Administration of pharmacological doses of 17α -ethinyloestradiol to rats markedly increases the rate of hepatic catabolism of lipoproteins that contain apo B and apo E (Chao *et al.*, 1979) and increases the

number of binding sites for these lipoproteins on liver membranes (Kovanen *et al.*, 1979*b*; Windler *et al.*, 1980). Rabbit liver membranes also contain LDL-binding sites, which can be regulated by dietary and drug treatments (Kovanen *et al.*, 1981; Floren *et al.*, 1981; Chao *et al.*, 1982*a,b*; Kroon *et al.*, 1982). Cholesterol feeding drastically decreases the catabolic rate of lipoproteins that contain apo B and apo E and decreases the number of hepatic binding sites for these lipoproteins (Kovanen *et al.*, 1981). As a result of this decrease in the catabolic rate, cholesterol-fed rabbits become severely hypercholesterolaemic.

LDL receptors from liver and other tissues share a number of common properties: (a) only lipoproteins that contain apo B or apo E bind to LDL receptors; (b) lipoproteins that contain apo E have a higher affinity than those that contain only apo B; (c) binding requires Ca^{+2} ; (d) lysine- and arginine-modified lipoproteins do not bind; and (e) the binding site is very sensitive to treatment with Pronase (Mahley & Innerarity, 1983). LDL receptors from different species share a considerable degree of immunological cross-reactivity. Rabbit antibodies prepared against the bovine adrenal LDL receptor cross-react with LDL receptors from

Abbreviations used: LDL, low-density lipoprotein; apo, apolipoprotein; β -VLDL, β -migrating very-low-density lipoprotein; SDS, sodium dodecyl sulphate.

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dog liver and adrenal gland and with LDL receptors from oestrogen-treated-rat liver (Beisiegel *et al.*, 1981a). However, there are also structural differences between LDL receptors, as shown by the finding that these antibodies do not react with rabbit LDL receptors (Beisiegel *et al.*, 1981a). Furthermore, a monoclonal antibody prepared against the bovine adrenal LDL receptor reacts only with the human and bovine LDL receptors, and not with receptors from the mouse, rat, Chinese hamster, rabbit or dog (Beisiegel *et al.*, 1981b).

In the current study we have characterized and compared LDL receptors from the rat and rabbit liver and adrenal glands, and from the bovine adrenal cortex. These studies were made possible because of the development of blotting techniques to detect LDL receptors bound to nitrocellulose paper (Daniel *et al.*, 1983). In this method solubilized membrane proteins are separated by SDS/polyacrylamide-gel electrophoresis and are transferred electrophoretically to nitrocellulose paper. LDL receptors bound to nitrocellulose paper retain their ability to bind lipoproteins and can be detected with ^{125}I -labelled antibodies to bound lipoproteins, or, as we have done in the present study, by binding ^{125}I -labelled lipoproteins directly to the receptors. The latter method avoids the use of specific antibodies and is extremely sensitive in that nanogram quantities of LDL receptors can be detected.

Experimental

Materials

We obtained bovine serum albumin (fraction V), 17α -ethinyloestradiol, phenylmethanesulphonyl fluoride, Tris maleate and octyl β -D-glucoside from Sigma, suramin from FBA Pharmaceuticals, Na^{125}I (14–17 mCi/ μg) from Amersham, bovine adrenal glands from a local slaughterhouse, and nitrocellulose BA85 paper (0.45 μm pore size) from Schleicher and Schuell.

Lipoproteins

Human LDL ($1.019 < d < 1.063 \text{ g/ml}$) and rabbit β -VLDL ($d < 1.006 \text{ g/ml}$) were isolated by ultracentrifugation (Kroon & Seidenberg, 1983) at 5°C in a Ti60 rotor in a Beckman preparative ultracentrifuge and were dialysed extensively against 0.15 M-NaCl/0.3 mM-EDTA, pH 7.4. LDL and β -VLDL were radiolabelled with ^{125}I by the ICI method as previously described (Brown & Goldstein, 1974). Specific radioactivities were 300–400 c.p.m./ng of protein for both LDL and β -VLDL. ^{125}I -labelled β -VLDL was used within 3 days of preparation, and ^{125}I -labelled LDL within

1 week. ^{125}I -labelled LDL was filtered through a Millipore filter (0.45 μm pore size) before use.

Animals, diets and drugs

Male Sprague–Dawley rats were obtained from Charles River. Rats used for liver membrane preparations weighed approx. 500 g at the start of the experiment. Rats used for adrenal membrane preparations weighed 150–200 g. 17α -Ethinyloestradiol was dissolved in propylene glycol (5 mg/ml) and was administered subcutaneously at a dose of 5 mg/kg body wt. every 24 h for 3 days.

Male New Zealand White rabbits obtained from HARE (Hewitt, NJ, U.S.A.) were maintained on a Purina rabbit chow diet or on milled Purina rabbit chow supplemented with cholesterol (0.5%, w/w) and corn oil (10%, v/w). Cholesterol-fed rabbits were maintained on the cholesterol-rich diet for at least 2 months.

Preparation of solubilized membranes

Rat and rabbit liver membranes were prepared as described by Kovanen *et al.* (1979b).

Adrenal membranes were prepared as described previously (Schneider *et al.*, 1979). Whole rat and rabbit adrenal glands (cortex and medulla) were used. For bovine adrenal preparations only the cortex was used.

The membrane pellets were solubilized as described by Schneider *et al.* (1979). Solubilized preparations were stored at -70°C .

Analyses

Protein was determined by the Peterson (1977) modification of the Lowry assay. Samples containing β -octyl glucoside were precipitated with trichloroacetic acid before assay (Peterson, 1977).

Electrophoresis

One-dimensional SDS/polyacrylamide-gel electrophoresis was performed on 1.5 mm-thick 5% or 7% polyacrylamide slab gels in the presence of 0.1% SDS as described by Laemmli (1970). Solubilized membrane preparations (20–100 μg of protein) were applied in 45 μl of 3.3 mM-Tris/HCl buffer, pH 6.8, containing 1.3% SDS, 13% sucrose and 0.03% (w/v) Bromophenol Blue. To retain the original structure as much as possible, no reducing agent was added and the samples were not heated. Electrophoresis was performed at room temperature at 130 V for 4–5 h. Gels were calibrated with M_r standards (myosin, 200 000; β -galactosidase, 116 000; phosphorylase *b*, 94 000; bovine serum albumin, 68 000; ovalbumin, 43 000) purchased from Bio-Rad, which were applied to one side of each SDS gel.

Electrophoretic blotting of proteins on to nitrocellulose

Proteins were transferred from SDS slab gels on to nitrocellulose paper (Burnette, 1981) by using a Trans-Blot Cell (Bio-Rad). Each slab gel was placed on a sheet of pre-wetted nitrocellulose. A sheet of filter paper and a Bio-Rad foam pad were placed on each side to prepare the 'sandwich' for electrophoresis. The electrode buffer consisted of 25 mM-Tris, pH 8.3, 192 mM-glycine and 20% (v/v) methanol. Electrophoresis was run with cooling at 4°C, at 30 V for 16 h and subsequently at 50 V for an additional 1 h.

Incubation of nitrocellulose with ^{125}I -labelled lipoproteins

The region of the nitrocellulose paper which contained the samples was incubated for 30 min at 40°C in 10 mM-Tris/HCl (pH 8)/150 mM-NaCl/0.5 mM- CaCl_2 (buffer A) containing 50 mg of bovine serum albumin/ml, followed by incubation for 90 min at room temperature in the same buffer containing 2×10^6 c.p.m. of ^{125}I -labelled human LDL or rabbit β -VLDL/ml in the presence or absence of 10 mM-EDTA or 3.5 mM suramin. Each nitrocellulose transfer was prepared in duplicate, one for incubation with 10 mM-EDTA, and one without it. Incubations were performed in a volume of 20 ml in heat-sealed plastic bags (Seal-N-Save; Sears Roebuck). The nitrocellulose sheets were then washed for 10 min in 200 ml of buffer A, followed by two 20 min washes in the same buffer. Each of the above incubations and washing steps were performed with rocking. After the last wash, the sheet was blotted dry, wrapped in Saran Wrap (Dow Chemical Co.) and exposed at -70°C to Kodak XR film for 1–16 h with Cronex Lightening Plus enhancing screens (E. I. DuPont). M_r standards were detected by staining with Coomassie Blue (Burnette, 1981) or by incubating the standards strip as described for the sample strip, with gelatin instead of bovine serum albumin in the blocking step. In the latter method, ^{125}I - β -VLDL was found to bind to the standard protein bands, and could be detected by autoradiography.

Results*Detection of LDL receptors by lipoprotein blotting*

The bovine adrenal cortex contains a high concentration of LDL receptors. These receptors bind both human LDL (Kovanen *et al.*, 1979a; Melio *et al.*, 1980) and rabbit β -VLDL (P. A. Kroon & Y.-S. Chao, unpublished work). When membranes prepared from the bovine adrenal cortex were solubilized in 40 mM- β -octyl glucoside and separated by SDS/polyacrylamide-gel electrophoresis, several bands were seen in the Coomassie-Blue-stained gel (Fig. 1, lane 1). Most of these

Fig. 1. SDS/polyacrylamide-gel electrophoresis and lipoprotein blotting of LDL receptors from bovine adrenal cortex

Solubilized adrenal-cortex membrane proteins (84 μg of protein/lane) were subjected to electrophoresis in 7% acrylamide slab gels containing 0.1% SDS. The gel strip in lane 1 was stained with Coomassie Blue. Proteins in lanes 2–6 were transferred electrophoretically to nitrocellulose paper and were incubated with the following: lane 2, ^{125}I -LDL; lane 3, ^{125}I -LDL with 10 mM-EDTA; lane 4, ^{125}I -LDL with 7 mM-suramin; lane 5, ^{125}I - β -VLDL; lane 6, ^{125}I - β -VLDL with 10 mM-EDTA. Each incubation solution contained ^{125}I -labelled lipoprotein (300–400 c.p.m./ng). The dried papers were used for autoradiography.

had M_r values less than 116000. To locate the receptor, proteins were transferred electrophoretically from the SDS/polyacrylamide gel to a sheet of nitrocellulose paper. After transfer, the nitrocellulose sheet was incubated in buffer containing 5% (w/v) bovine serum albumin to block additional protein-binding sites, and was subsequently incubated with ^{125}I -LDL or with ^{125}I - β -VLDL, in the presence of 0.5 mM- CaCl_2 , and then washed. Autoradiography showed a single major band for both the ^{125}I -LDL (lane 2) and ^{125}I - β -VLDL incubation (lane 5), corresponding to M_r 132000. When the nitrocellulose transfers were incubated in identical ^{125}I -lipoprotein solutions to which EDTA had been added, this band was not observed (lanes 3 and 6). Suramin, which is known to prevent binding of ^{125}I -LDL to receptors (Schneider *et al.*, 1982), also prevented the binding of ^{125}I -LDL to the nitrocellulose transfer (lane 4). The binding of ^{125}I -LDL and ^{125}I - β -VLDL is therefore characteristic of LDL-receptor interactions. These experiments show that LDL receptors can be detected directly in solubilized membrane preparations by using ^{125}I -labelled lipoproteins which contain either apo B (LDL) or

apo B and apo E (β -VLDL). Of these two lipoproteins, ^{125}I -labelled β -VLDL binds to a greater extent under the conditions used. This is apparent from film exposure times, which are generally 3–6 times shorter for nitrocellulose sheets incubated with ^{125}I - β -VLDL than with ^{125}I -LDL. This difference is in part attributable to the different affinity of apo B- and apo E-containing lipoproteins for the LDL receptor. In addition to the major band, two minor bands were observed (lanes 2 and 5), one above the M_r -200000 standard, and one at M_r 125000. Like the major band, the minor bands were not observed in the presence of EDTA and of suramin.

In this study the binding and EDTA-sensitivity of binding were determined on separate nitrocellulose sheets. We have found, however, that nitrocellulose sheets that have been incubated with ^{125}I -labelled LDL or β -VLDL in the presence of EDTA and processed for autoradiography can be re-used for binding experiments after incubation with a CaCl_2 -containing buffer.

Characterization of rat LDL receptors

Previous studies have shown that the number of rat liver LDL receptors is markedly increased when rats are treated with pharmacological doses of 17α -ethinyloestradiol (Kovanen *et al.*, 1979b; Windler *et al.*, 1980). When solubilized liver membranes from normal rats were subjected to lipoprotein blotting with ^{125}I - β -VLDL, in the presence of CaCl_2 , a receptor band with M_r 145000 was observed (Fig. 2, lane 1). This band was not observed in the presence of EDTA (lane 2). Under the same conditions, solubilized liver membranes from rats that had been treated with 17α -ethinyloestradiol displayed a receptor band with the same electrophoretic mobility, but with a much greater intensity (lane 3). A more careful comparison of the M_r values of the hepatic LDL receptors from normal and oestrogen-treated rats was made by mixing solubilized bovine adrenal and rat liver membranes before electrophoresis on a 5%-polyacrylamide gel. This gives an internal standard for comparison of M_r values, such that differences in M_r of approx. 1500 can be detected. Under these conditions there was no detectable difference in the mobility of LDL receptors from oestrogen-treated- and control-rat livers (results not shown).

The lipoprotein-blotting technique was also used to detect LDL receptors in rat adrenal homogenates. These experiments revealed a receptor (lane 4, Fig. 2) which migrated slightly faster than the rat liver receptor. The difference in migration was quantified by using the bovine adrenal LDL receptor as an M_r reference in the same lane. By

Fig. 2. Lipoprotein blotting of LDL receptors from normal and oestrogen-treated-rat livers and from rat adrenal glands

Solubilized membrane proteins were subjected to electrophoresis in 7%-acrylamide slab gels containing 0.1% SDS. Proteins were transferred electrophoretically to nitrocellulose paper. Lanes 1, 3 and 4 were incubated with ^{125}I - β -VLDL, and lane 2 with ^{125}I - β -VLDL and 10mM-EDTA. Incubation solutions contained 2×10^6 c.p.m. of ^{125}I - β -VLDL (300–400 c.p.m./ng). The dried nitrocellulose papers were used for autoradiography. The following solubilized membrane preparations were used: lanes 1 and 2, normal rat liver (46 μg of protein); lane 3, oestrogen-treated-rat liver (59 μg of protein); lane 4, rat adrenal glands (76 μg of protein).

this procedure, the rat adrenal LDL receptor was shown to have M_r 142000.

Both the liver from oestrogen-treated rats and the adrenals from normal rats contained minor components which bound ^{125}I - β -VLDL in the presence of Ca^{2+} . Similar bands were observed for the bovine adrenal preparations (Fig. 1), although the rat adrenal preparation contained relatively more of the lower- M_r component.

Characterization of rabbit LDL receptors

Lipoprotein blots of solubilized rabbit liver membrane proteins are shown in Fig. 3 for chow-fed rabbits (lanes 1–4) and for cholesterol-fed rabbits (lane 5). Livers from rabbits fed on a chow diet have a receptor with M_r 128000, which binds both ^{125}I -LDL (lane 1) and ^{125}I - β -VLDL (lane 3) in the presence of Ca^{2+} . Binding is abolished by EDTA (lanes 2 and 4). This receptor is not observed when liver membranes from a cholesterol-fed rabbit are used (lane 5), consistent with the down-regulation of liver LDL receptors in cholesterol-fed rabbits (Kovanen *et al.*, 1981). The rabbit adrenal gland contains an LDL receptor (lane 6)

Fig. 3. Lipoprotein blotting of LDL receptors from chow- and cholesterol-fed rabbit livers and chow-fed rabbit adrenal glands

Solubilized membrane proteins were subjected to electrophoresis in 7%-acrylamide slab gels containing 0.1% SDS. Proteins were transferred electrophoretically to nitrocellulose paper and were incubated with 2×10^6 c.p.m. of ^{125}I -LDL or ^{125}I - β -VLDL (300–400 c.p.m./ng). The dried nitrocellulose paper was used for autoradiography. Lanes 1–4, solubilized liver membranes from chow-fed rabbits (65 μg of protein per lane), incubated with ^{125}I -LDL (lane 1), ^{125}I -LDL + 10 mM-EDTA (lane 2), ^{125}I - β -VLDL (lane 3), ^{125}I - β -VLDL + 10 mM-EDTA (lane 4); lane 5, solubilized liver membranes from cholesterol-fed rabbits (65 μg of protein) incubated with ^{125}I -LDL; lane 6, solubilized adrenal membranes from chow-fed rabbits (38 μg of protein), incubated with ^{125}I -LDL.

with an M_r value close to that of the liver receptor. Close inspection, however, shows that the rabbit adrenal receptor has a slightly lower mobility than that of the liver receptor. This difference has been quantified by including solubilized rat liver membrane proteins with the rabbit proteins before electrophoresis on a 5%-polyacrylamide gel. Under these conditions, the rabbit adrenal receptor has an apparent M_r of 131 000, in good agreement with the value of 130 000 reported by Daniel *et al.* (1983).

Comparison of LDL receptors from the rat and rabbit liver and bovine adrenal cortex

To demonstrate the apparent M_r differences between the rabbit and rat liver LDL receptors (128 000 and 145 000 respectively) and the bovine adrenal-cortex LDL receptor (132 000), mixtures of homogenates from these tissues were subjected to lipoprotein blotting. The results of such experiments are shown in Fig. 4. Lane 1 shows LDL-receptor bands for a mixture of rat liver and bovine adrenal homogenates, and lane 2 for a mixture of rat and rabbit liver homogenates. These data

Fig. 4. Lipoprotein blotting of LDL receptors from rabbit liver and bovine adrenal receptors, with rat liver receptors added as an internal M_r marker

Solubilized membrane proteins were subjected to electrophoresis in 5%-acrylamide slab gels containing 0.1% SDS. Proteins were transferred electrophoretically to nitrocellulose paper and incubated with 2×10^6 c.p.m. of ^{125}I - β -VLDL (300–400 c.p.m./ng). The dried nitrocellulose paper was used for autoradiography. Lane 1, combined solubilized bovine adrenal membranes and oestrogen-treated-rat liver membranes; lane 2, combined solubilized rabbit liver membranes and oestrogen-treated-rat liver membranes.

clearly demonstrate that the rat liver LDL receptor is substantially larger than the bovine adrenal and rabbit liver LDL receptors.

Discussion

This paper describes the detection and characterization of liver and adrenal LDL receptors, in the rat and rabbit, by lipoprotein blotting. In this method, crude membrane preparations are solubilized in β -octyl glucoside and are then subjected to SDS/polyacrylamide-gel electrophoresis. Electrophoresis is performed in the absence of thiol reducing agents and without heating the samples. Proteins are transferred electrophoretically to nitrocellulose paper, which is incubated with ^{125}I -labelled human LDL or rabbit β -VLDL. Bound lipoproteins are subsequently detected by autoradiography.

Several lines of evidence show that the proteins which bind LDL and β -VLDL correspond to the LDL receptor. First, lipoprotein binding displays a specificity which is characteristic of the LDL receptor in intact membranes, in that they bind

lipoproteins that contain apo B (LDL) and lipoproteins that contain apo B and apo E (β -VLDL), and that binding of LDL required Ca^{2+} , and is inhibited by suramin, a polycyclic hydrocarbon which is known to inhibit the binding of LDL to LDL receptors (Schneider *et al.*, 1982). In other experiments we have also shown that unlabelled LDL and β -VLDL inhibit the binding of the corresponding ^{125}I -labelled lipoproteins (results not shown). Second, there is a correspondence between the amount of ^{125}I -lipoprotein bound to the nitrocellulose paper and the number of hepatic LDL receptors after treatment of the animals with different diets and drugs. Thus, when rabbits are fed on a cholesterol-supplemented diet, binding studies show almost a complete absence of LDL-receptor-binding sites on liver membranes (Kovanen *et al.*, 1981), and no LDL binding is detected by lipoprotein blotting. Furthermore, when rats are treated with pharmacological doses of 17α -ethinyloestradiol, the number of LDL-receptor-binding sites in the liver increases more than 10-fold (Kovanen *et al.*, 1979b; Windler *et al.*, 1980), and a corresponding increase is seen in the amount of ^{125}I - β -VLDL bound to the receptor band on the nitrocellulose paper. Our studies therefore provide both biochemical and physiological evidence that identifies the hepatic and adrenal proteins detected by lipoprotein blotting as classical LDL receptors.

The sensitivity of the lipoprotein-blotting techniques is demonstrated by the fact that LDL receptors can be detected in liver membranes from untreated rats and rabbits. Rabbit liver membranes bind approx. 30 ng of rabbit LDL/mg of membrane protein to EDTA-sensitive binding sites (Kita *et al.*, 1981). The amount of solubilized membrane protein used in each gel lane was derived from about 300 μg of membrane protein; this is able to bind approx. 9 ng of LDL protein. If we assume that human and rabbit LDL bind similarly, that one LDL particle binds per receptor molecule, and that the LDL protein component has M_r approx. 4×10^5 , we have detected approx. 3 ng of receptor. For comparison, the reported sensitivities for the detection of LDL receptors by immunoblotting (Beisiegel *et al.*, 1982) and ligand blotting (Daniel *et al.*, 1983) are 1 μg and 0.25 μg respectively. The reason for this difference in sensitivity is not clear at present.

The M_r determined for the bovine adrenal LDL receptor by lipoprotein blotting is 132 000, which is substantially less than the value of 160 000 found for the purified receptor (Schneider *et al.*, 1982). The same observation was made by Daniel *et al.* (1983). These differences can be attributed to the fact that the M_r of the purified receptor was determined in the presence of a reducing agent, 2-

mercaptoethanol, whereas the lipoprotein-blotting experiments were performed in the absence of a reducing agent. The presence of reducing agents abolishes LDL-receptor binding. Reduction of disulphide bonds most likely unfolds the receptor completely in the presence of SDS, whereas the non-reduced receptor is not completely unfolded. This is consistent with the fact that the intrinsic viscosities of unreduced proteins in SDS are considerably smaller than the intrinsic viscosities of the corresponding reduced proteins (Fish *et al.*, 1970).

In the present study we show that the M_r values of the bovine and rabbit LDL receptors are very similar. In contrast, the rat LDL receptors have a substantially higher M_r . Since the removal of sialic acid residues from the bovine adrenal receptors by treatment with neuraminidase results in a decrease in M_r of 7000–11 000 (Schneider *et al.*, 1982), it appeared possible that bovine and rabbit LDL receptors may contain a smaller number of sialic acid residues than do rat LDL receptors. However, when bovine adrenal and rat liver LDL receptors were treated with neuraminidase before electrophoresis, the M_r of both receptors decreased by about 10 000 (results not shown). Sialic acid residues therefore do not appear to be responsible for the difference in M_r between these receptors. Differences in the size of the protein moiety, in the number of oligosaccharide chains, or in the conformation of the receptor resulting from an altered combination of internal disulphide bonds cannot be ruled out.

The differences in M_r between the adrenal and liver LDL receptors are small but reproducible. These differences could only be measured with certainty when a reference LDL-receptor M_r marker was included with each of the samples before electrophoresis; for rat receptors the bovine adrenal LDL receptor was used as a reference, and for the rabbit receptors the rat liver LDL receptor was used as a reference. Although the nature of the differences in M_r is not clear, they are most likely at the level of glycosylation. For the rabbit, this is well founded, since the adrenal and liver LDL receptors have been shown to correspond to the same gene product (Kita *et al.*, 1981). This conclusion is based on studies of the Watanabe heritable hyperlipaemic rabbit, which has a single gene defect resulting in the absence of detectable amounts of receptor in the liver and adrenal glands (Kita *et al.*, 1981).

Finally, the question has been raised as to whether the LDL receptor of oestradiol-treated rat livers is the same as that normally expressed in rat livers or whether it is the product of a distinct gene induced by pharmacological doses of oestradiol (Kovanen *et al.*, 1979b; Windler *et al.*, 1980). Our

data show that the M_r of the LDL receptors from oestradiol-treated rat livers is indistinguishable from that of control rat livers, consistent with the concept that the liver LDL receptors from control and oestradiol-treated rats are the product of the same gene.

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