Modulation of the low-density-lipoprotein-receptor-related protein and its relevance to chylomicron-remnant metabolism

Attila SZANTO,* Santhira BALASUBRAMANIAM,*† Paul D. ROACH† and Paul J. NESTEL‡

*Lipid Research Department, University of New South Wales School of Medicine, St. Vincent's Hospital, Sydney, N.S.W. 2010, and †CSIRO Division of Human Nutrition, Adelaide, S.A. 5000, Australia

Hepatic levels of the low-density-lipoprotein (LDL)-receptor-related protein (LRP) and the LDL receptor were measured in rats subjected to treatments known to affect the expression of the LDL receptor. Propylthiouracil decreased both hepatic LRP and LDL receptor expression by 30–40%. Thyroxine treatment increased LDL receptor levels by 3-fold without altering LRP levels. In contrast, 17 α -ethinyloestradiol decreased LRP by 50%, whereas the LDL receptor was increased 5-fold. Plasma chylomicrons and their remnants were decreased to insignificant levels with this treatment. In rats fed with cholesterol there was a significant increase in these particles in plasma (1.21 ± 0.4 versus 5.71 ± 0.4 mg/dl), whereas the expression of LRP was unaltered. In Watanabe heritable hyperlipidaemic and cholesterol-fed rabbits, in which the LDL receptor expression is absent or decreased, the expression of LRP was not significantly different from that in normal rabbits. These results suggest that the expression of hepatic LRP can be modulated by changes in the hormonal status of the rat and that this modulation is not tightly co-ordinated with that of the LDL receptor. Moreover, LRP does not appear to have a significant role in chylomicron-remnant clearance, whereas the LDL receptor is actively involved in this process.

INTRODUCTION

The low-density-lipoprotein (LDL)-receptor-related protein (LRP) is a glycoprotein located on the surface and intracellular membranes of a wide variety of cells and tissues (Herz *et al.*, 1988). It has an apparent molecular mass of 600 kDa, and during its migration to the cell surface LRP undergoes physiological cleavage into two subunits of 515 kDa and 85 kDa (Herz *et al.*, 1990a). These subunits remain non-covalently associated to form the functional LRP molecule. By sequence analysis, LRP has now been shown to be the α_2 -macroglobulin receptor (Strickland *et al.*, 1990; Kristensen *et al.*, 1990).

As its name implies, LRP is structurally and functionally related to the LDL receptor. The former has several repeats of the ligand-binding domains of the latter, which can undergo acid-dependent conformational changes during endocytosis to eject ligands within the endosomes before recycling to the plasma membrane (Herz *et al.*, 1990b). In addition LRP, like the LDL receptor, can bind apolipoprotein-E-enriched lipoproteins and appears to mediate their internalization and lysosomal degradation (Kowal *et al.*, 1989, 1990). There is evidence that the LDL receptor participates in clearing chylomicron remnants from the circulation (Choi *et al.*, 1991; Jackle *et al.*, 1992), and circumstantial evidence gathered in recent years suggests that LRP is a candidate for clearance of chylomicron remnants by the LDL-receptor-independent pathway (Beisiegel *et al.*, 1991; Husain *et al.*, 1991). The LDL receptor is modulated by a variety of dietary and hormonal factors, and its degree of expression has been shown to determine plasma LDL levels (Soutar & Knight, 1990). Because of its structural and functional resemblance to the LDL receptor, it could be postulated that the expression of LRP is also modulated by some of these factors and that any alteration in its expression would also be expected to influence the plasma level of chylomicron-remnant particles.

In the present work, the hepatic levels of LRP and the LDL

receptor were measured in rats subjected to treatments known to affect the expression of the LDL receptor to see whether the expression of LRP could also be modulated and, if so, whether such modulation could influence chylomicron-remnant levels in plasma. We also determined the levels of LRP in normal rabbits, in cholesterol-fed rabbits and in homozygous Watanabe heritable hyperlipidaemic (WHHL) rabbits, in which LDL receptors are absent. The results indicate that the expression of hepatic LRP in the rat is subject to hormonal modulation and that this modulation is not tightly co-ordinated with that of the LDL receptor. The results also suggest that the participation of LRP in chylomicron-remnant clearance is not rate-limiting and that the LDL receptor has a major role in chylomicron-remnant clearance from plasma.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 200–250 g were acclimatized to a 12 h-light/12 h-darkness cycle (lights on at 06:00 h) for at least 2 weeks before treatment and were fed *ad libitum* during all phases. Rats were administered 5 mg of 17 α -ethinyloestradiol/kg body wt. per day or 500 μ g of L-thyroxine/kg body wt. per day by subcutaneous injection. The diet of other rats was supplemented with 1% (w/w) propylthiouracil (PTU) or 1% (w/w) cholesterol in their chow (Bhattacharya *et al.*, 1986). All treatments were carried out for 7 days. Rats treated with L-thyroxine were hyperthyroid, with plasma concentrations of thyroxine averaging 159 ± 9 nmol/l (mean $\pm$ S.E.M.), and the rats treated with PTU were hypothyroid, with thyroxine levels of 7 ± 1 nmol/l, compared with 39 ± 4 nmol/l for normal rats. One group of three New Zealand White rabbits, each weighing 3 kg, was fed on a standard commercial rabbit pellet diet, and another group of three was fed on this diet supplemented with 1% (w/w) cholesterol for 7 days. Liver samples from homozygous WHHL

Abbreviations used: LDL, low-density lipoprotein; LRP, LDL-receptor-related protein; β VLDL, β -very-low-density lipoprotein; TRL, triacylglycerol-rich lipoprotein; PTU, propylthiouracil; WHHL, Watanabe heritable hyperlipidaemic.

† To whom correspondence should be addressed.

and normal rabbits were provided by Dr. John C. L. Mamo (University of Western Australia).

Preparation of liver membrane fractions and solubilization

A crude ultracentrifugal membrane fraction (8000–100 000 *g*) from liver was prepared as described by Kovanen *et al.* (1979) in 50 mM-Hepes (pH 7.4)/100 mM-NaCl/1 mM-phenylmethane-sulphonyl fluoride / 10 mM-*N*-ethylmaleimide / 10 mM-EDTA / 10 mM-EGTA as the homogenizing buffer. The membranes were suspended in 50 mM-Hepes/100 mM-NaCl, solubilized by adding an equal volume of 4% (w/v) SDS/20% (v/v) glycerol/0.005% (w/v) Bromophenol Blue, and protein was determined by the method of Lowry *et al.* (1951).

Quantification of LRP and the LDL receptor

The solubilized membrane proteins were subjected to electrophoresis on 3–12% linear gradient SDS/polyacrylamide gels as described by Lund *et al.* (1989) at 175 V for 3 h along with Rainbow molecular-mass markers (Pharmacia LKB, Uppsala, Sweden). The separated proteins were electrotransferred from the gels on to nitrocellulose membranes at 30 V for 16 h. The electroblots were incubated with $^{45}\text{Ca}^{2+}$ (2 mCi/l, 25 mCi/mg of Ca^{2+}) for 15 min and washed as described by Maruyama *et al.* (1984). They were then exposed to Kodak XAR-5 film for 16 h, and the bands corresponding to LRP and the LDL receptor were quantified with a scanning laser densitometer (LKB Ultrascan).

Preparation of lipoprotein fractions

Triacylglycerol-rich lipoproteins (TRL; *d* < 1.006) were prepared from rat plasma by ultracentrifugation at 100 000 *g* for 16 h at 18 °C. The floating TRL fraction was then overlaid with NaCl solution (1.006 g/ml) and ultracentrifuged at 100 000 *g* for 1 h. The floating fraction was taken to be the S_{100} fraction, and consisted mainly of chylomicrons and their remnants. Cholesterol in whole plasma and in the TRL and S_{100} fractions was measured by using enzymic kits (Boehringer-Mannheim, Mannheim, Germany). β -Very-low-density lipoprotein (β VLDL) was prepared from cholesterol-fed rabbits as described by Kowal *et al.* (1989).

Antibodies and β VLDL ligand binding

Polyclonal anti-LDL-receptor antibodies were prepared by injecting purified bovine adrenal-cortex LDL receptors into New Zealand White rabbits. Polyclonal anti-LRP antibodies directed against the LRP tail (LRP-85) were kindly donated by Dr. Keith K. Stanley. The LRP 515 subunit was detected and quantified with biotinylated β VLDL enriched with human apolipoprotein E by modification of the procedure described by Kowal *et al.* (1990). Streptavidin-horseradish peroxidase and the enhanced chemiluminescence (ECL) procedure (Amersham International, U.K.) were used instead of ^{125}I -labelled streptavidin.

RESULTS

LRP and the LDL receptor from rat liver membranes were detected by ligand blotting with $^{45}\text{Ca}^{2+}$ (Fig. 1). Only the 515 kDa subunit of the LRP was seen, as the 85 kDa subunit does not bind Ca^{2+} . The LDL receptor band was at a position corresponding to 130 kDa, as the electrophoresis was done under non-reducing conditions. The presence of the LRP 515 band was confirmed with biotinylated β VLDL enriched with human apoE, and the LRP 85 band by using the polyclonal antibody (anti-

LRP 85), which did not cross-react with the LDL receptor. The presence of the LDL receptor band was also confirmed by using the polyclonal anti-LDL-receptor antibody. This antibody did not cross-react with LRP. The intensity of the LRP and the LDL receptor bands obtained with the $^{45}\text{Ca}^{2+}$ ligand-blotting method showed a linear response to the amount of protein loaded on to the gel. The linearity was maintained up to 150 μg of solubilized membrane protein loaded. In all the experiments in this study 100 μg of protein was used. The reason for using the $^{45}\text{Ca}^{2+}$ ligand-blotting procedure was that it is easy to perform, rapid and sensitive, allowing the observation and quantification of both proteins simultaneously.

Fig. 1. Detection of LRP and the LDL receptor in rat liver by ligand blotting with $^{45}\text{Ca}^{2+}$.

Microsomal membranes prepared from rat liver cells were solubilized in SDS and subjected to SDS/PAGE (3–12% linear gradient, under non-reducing conditions) and to electrophoretic transfer to nitrocellulose membranes. The nitrocellulose blots were incubated with $^{45}\text{Ca}^{2+}$ and autoradiographed as described in the text. Rainbow molecular-mass standards (myosin, 200 kDa; phosphorylase *b*, 97 kDa; BSA, 69 kDa; ovalbumin, 46 kDa; carbonic anhydrase, 30 kDa; trypsin inhibitor, 22 kDa; lysozyme, 14 kDa) were run on adjacent lanes.

Table 1. Hepatic expression of LRP and the LDL receptor in rats subjected to various treatments

Rats weighing 250 g were injected daily with oestradiol (5 mg/kg body wt.) or thyroxine (0.5 mg/kg body wt.) subcutaneously for 7 days or given diet supplemented with PTU (1%, w/w) for 7 days. Each treatment had its own controls. Membranes prepared were solubilized, and LRP and the LDL receptor were separated and quantified as described in the text. The results are expressed as means $\pm$ S.E.M., and six rats were used for each treatment. Peak areas, measured in arbitrary units, were expressed as a percentage of the values obtained with control animals. Statistical analysis of differences from control ($*P < 0.01$) was done by Student's *t* test.

	Expression (arbitrary units)	
	LRP	LDL receptor
Control	100	100
Oestradiol	43 $\pm$ 4*	496 $\pm$ 21*
Thyroxine	98 $\pm$ 3	298 $\pm$ 25*
PTU	72 $\pm$ 4*	60 $\pm$ 12*

Fig. 2. LRP and the LDL receptor in control (a) and oestradiol-treated (b) rats

LRP and the LDL receptor were separated from solubilized liver membranes and quantified as described in the text.

Fig. 3. LRP and the LDL receptor in normal (a) and homozygous WHHL (b) rabbits

LRP and the LDL receptor were separated from solubilized liver membranes and quantified as described in the text.

The hepatic expression of LRP and the LDL receptor in rats treated with oestradiol, L-thyroxine and PTU are shown in Table 1. Fig. 2 illustrates a typical example of an autoradiograph of LRP and LDL receptor bands in a control and an oestradiol-treated animal. Treatment with oestradiol decreased the LRP band by 50% and increased the LDL receptor band by 5-fold. Treatment with PTU, to render the rats hypothyroid, also decreased expression of the hepatic LRP band by 30%, with similar effects on the LDL receptor (–40%). Hyperthyroidism, as obtained with injections of L-thyroxine, had no effect on LRP, but increased the LDL receptor expression by 3-fold. Fig. 3

Table 2. Effects of cholesterol feeding and oestradiol treatment on plasma levels of TRL and chylomicron remnants

Rats weighing 250 g were given a diet supplemented with cholesterol or were injected with oestradiol as described in the legend for Table 1. Plasma collected at the end of the 7-day treatment period was separated into TRL fraction ($d < 1.006$) and chylomicron-remnant fraction ($S_r > 60$) as described in the text, and cholesterol levels in both these fractions and in whole plasma were determined. Hepatic LRP was quantified as described previously. Each value is the mean $\pm$ S.E.M. for six rats. Statistical analysis of differences from control ($*P < 0.01$) was done by Student's t test.

Treatment	LRP (% of control)	Cholesterol (mg/dl)		
		Whole plasma	TRL fraction	$S_r > 60$ fraction
Control	100	73.3 ± 1.5	3.83 ± 0.08	1.21 ± 0.04
Oestradiol	$47 \pm 1^*$	$7.7 \pm 0.3^*$	$< 0.06^*$	$< 0.01^*$
Cholesterol	98 ± 2	$101 \pm 3^*$	$16.9 \pm 0.7^*$	$5.7 \pm 0.4^*$

shows the intensity of LRP and LDL receptor bands in normal and homozygous WHHL rabbits. The levels of LRP and the LDL receptor (expressed as a percentage of the value observed in normal rabbit) in homozygous WHHL rabbits were $97 \pm 4\%$ and $< 5\%$ respectively. In rabbits fed with cholesterol the corresponding values were $98 \pm 3\%$ and $43 \pm 5\%$.

Hepatic levels of LRP and the cholesterol concentration in plasma, TRL fraction and $S_r > 60$ fractions in control, oestradiol-treated and cholesterol-fed rats are shown in Table 2. In oestradiol-treated rats there was a significant decrease in plasma cholesterol and a complete depletion of cholesterol in both TRL and the $S_r > 60$ fractions. In cholesterol-fed rats, on the other hand, a significant increase in the cholesterol level in plasma and in both the lipoprotein fractions was observed. Although the TRL fraction includes chylomicrons, their remnants and VLDL, the $S_r > 60$ fraction represents mainly a spectrum of chylomicron-remnant particles, and hence the cholesterol level in this fraction reflects the chylomicron-remnant level in plasma.

DISCUSSION

Our results clearly demonstrate that the hepatic expression of LRP can be modulated by the hormonal status of the rat. Like the LDL receptor, LRP is significantly down-regulated in the hypothyroid state. However, during oestradiol treatment, unlike the LDL receptor, which is markedly increased, LRP is decreased by half, and hence its modulation is not co-ordinated with that of the LDL receptor. This lack of co-ordination is also seen in hyperthyroid rats, in which there is an increase in the expression of the LDL receptor, but no alteration in the expression of LRP. It is possible that LRP is expressed normally at its maximum potential and cannot be induced further by any treatment. This would be in agreement with the structure of the LRP promoter, which resembles that of a house-keeping gene (Kutt *et al.*, 1989). The failure of LRP to respond to cholesterol feeding in the present study is in agreement with the absence of a sterol-regulatory element in the promoter region of its gene (Kutt *et al.*, 1989). Previous studies have suggested that hepatic clearance of chylomicron remnants is not subject to modulation (Arbeeny & Rifci, 1984; Nagata *et al.*, 1988). Our observation that the expression of LRP can be modulated raises the possibility that, if LRP contributes to chylomicron-remnant clearance, then its participation in this process is not rate-limiting.

LRP has recently been implicated as the receptor responsible for chylomicron-remnant clearance by the LDL-receptor-

independent pathway (Hussain *et al.*, 1991). Studies by Choi *et al.* (1991), using anti-LDL-receptor antibody to block the LDL receptor, established that about 50% of chylomicron remnants are cleared from plasma by the LDL-receptor-independent pathway. In our present studies chylomicron-remnant particles failed to accumulate in the plasma of oestradiol-treated rats, despite a decrease in the expression of LRP by half (Table 2). This would suggest either that LRP is not directly involved in the regulation of remnant clearance, or that its involvement in this clearance process is masked by the dramatic increase in the LDL receptor expression during this treatment. However, the total depletion of chylomicron-remnant particles from the circulation as a consequence of increased LDL receptor expression suggests that the LDL receptor has a regulatory role in remnant clearance. In cholesterol-fed rats, where the LDL receptor is down-regulated (Erickson *et al.*, 1989), our studies clearly show that chylomicron-remnant particles accumulate in the plasma, whereas the expression of LRP is not significantly altered (Table 2). This is consistent with our suggestion that the participation of LRP is not rate-limiting in chylomicron-remnant clearance.

Although the LDL receptor can clear chylomicron remnants from the circulation under normal circumstances, another receptor must assume this function when LDL receptors are defective or suppressed if chylomicron-remnant clearance is to remain normal. In our studies we have demonstrated that in cholesterol-fed rabbits, when the LDL receptor expression is partially suppressed (see the Results section), and in WHHL rabbits, where it is completely suppressed, the expression of LRP is unaltered (Fig. 3). In both these situations Mamo *et al.* (1991) and Bowler *et al.* (1991) have convincingly demonstrated that chylomicron-remnant clearance is progressively reduced. Taken together, these observations strengthen the view that LRP does not regulate chylomicron-remnant removal, even in situations where the LDL receptor expression is decreased to insignificant levels, because it does not up-regulate to compensate for this, thereby delaying remnant clearance. What clearly emerges from our studies is that the enhancement of the expression of the LDL receptor accelerates chylomicron-remnant clearance and that its suppression delays remnant clearance, thus suggesting that the LDL receptor regulates remnant clearance from plasma.

We thank Dr. Keith K. Stanley (Heart Research Institute), Professor Leon A. Simons from our Department, and Dr. John C. L. Mamo

(University of Western Australia) for their helpful discussions, and Michael Edwards for excellent technical assistance.

REFERENCES

- Arbeeny, C. M. & Rifci, V. A. (1984) *J. Biol. Chem.* **259**, 9662–9666
- Beisiegel, U., Weber, W. & Bengtsson-Olivecrona, G. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8342–8346
- Bhattacharya, S., Balasubramaniam, S. & Simons, L. A. (1986) *Biochem. J.* **234**, 493–496
- Bowler, A., Redgrave, T. G. & Mamo, J. C. L. (1991) *Biochem. J.* **276**, 381–386
- Choi, S. Y., Fong, L. G., Karvan, M. J. & Cooper, A. D. (1991) *J. Clin. Invest.* **88**, 1173–1181
- Erickson, S. K., Jaecckle, S., Lear, S. R., Brady, S. M. & Havel, R. J. (1989) *J. Lipid Res.* **30**, 1763–1771
- Herz, J., Hamann, U., Rogne, S., Myklbost, O., Gausepohl, H. & Stanley, K. K. (1988) *EMBO J.* **7**, 4119–4127
- Herz, J., Kowal, R. C., Goldstein, G. L. & Brown, M. S. (1990a) *EMBO J.* **9**, 1769–1776
- Herz, J., Kowal, R. C., Ho, Y. K., Brown, M. S. & Goldstein, J. L. (1990b) *J. Biol. Chem.* **265**, 21355–21362
- Hussain, M. M., Maxfield, F. R., Mas-Oliva, J., Tabas, I., Ji, Z. S., Innerarity, T. L. & Mahley, R. W. (1991) *J. Biol. Chem.* **266**, 13936–13940
- Jackle, S., Rinninger, F., Greeve, J., Greten, H. & Windler, E. (1992) *J. Lipid Res.* **33**, 419–429
- Kovanen, P. T., Brown, M. S. & Goldstein, J. L. (1979) *J. Biol. Chem.* **254**, 11367–11373
- Kowal, R. C., Herz, J., Goldstein, J. L., Esser, V. & Brown, M. S. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5810–5814
- Kowal, R. C., Herz, J., Weisgraber, K. H., Mahley, R. W., Brown, M. S. & Goldstein, J. L. (1990) *J. Biol. Chem.* **265**, 10771–10779
- Kristensen, T., Moestrup, S. K., Gliemann, J., Bendtsen, L., Sand, O. & Sottrup-Jensen, L. (1990) *FEBS Lett.* **276**, 151–155
- Kutt, H., Herz, J. & Stanley, K. K. (1989) *Biochim. Biophys. Acta* **1009**, 229–236
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Lund, H., Takahashi, K., Hamilton, R. L. & Havel, R. J. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 9318–9322
- Mamo, J. C. L., Bowler, A., Elsegood, C. L. & Redgrave, T. G. (1991) *Biochim. Biophys. Acta* **1081**, 241–245
- Maryauma, K., Kikawa, T. & Ebashi, S. (1984) *J. Biochem. (Tokyo)* **95**, 511–519
- Nagata, Y., Chen, J. & Cooper, A. D. (1988) *J. Biol. Chem.* **263**, 15151–15158
- Soutar, A. K. & Knight, B. L. (1990) *Br. Med. Bull.* **46**, 891–916
- Strickland, D. K., Ashcom, J. D., Williams, S., Burgess, W. H., Migliorini, M. & Argraves, W. S. (1990) *J. Biol. Chem.* **265**, 17401–17404

Received 21 April 1992/22 June 1992; accepted 1 July 1992