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CATABOLISM OF (^{125}I)LOW DENSITY LIPOPROTEINS IN ISOLATED RAT LIVER CELLS

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SUMMARY

Isolated rat hepatocytes and non-parenchymal liver cells bind and internalize rat (^{125}I)low density lipoproteins via a binding mechanism with affinity for both low density lipoproteins and high density lipoproteins. The cells degrade (^{125}I)low density lipoproteins at a rate which is 30-75% higher than can be attributed to fluid endocytosis. Leupeptin, an inhibitor of thiol proteases including cathepsin B, reduced degradation of (^{125}I)low density lipoproteins with about 15-20% in both hepatocytes and non-parenchymal liver cells. Subcellular fractionation by means of sucrose gradient centrifugation of hepatocyte homogenates suggested that low density lipoproteins taken up *in vivo* accumulated in lysosomes. Low density lipoproteins taken up by hepatocytes *in vitro* were distributed in the gradient similarly to the plasma membrane marker enzyme 5'-nucleotidase.

INTRODUCTION

Both low density and high density lipoproteins are taken up and catabolized in the rat liver (1,2) but the rate of uptake is low compared to the very efficient uptake of chylomicron remnants (3). HDL provide the steroid producing organs with cholesterol (4). The physiological importance of LDL in the lipoprotein metabolism of the rat is presently not clear. The mechanism by which the liver takes up and catabolizes LDL is unknown. Fluid endocytosis has been proposed to be responsible (5), but adsorptive endocytosis is more likely(6,7,8). We have previously shown that HDL bind to rat hepatocytes and non-parenchymal liver cells through a saturable mechanism which differs from the LDL-receptor in fibroblasts (9,10).This paper reports data on the binding, uptake and degradation of rat LDL in hepatocytes in heaptocytes and non-parenchymal liver cells.

Abbreviations

EDTA: ethylenediamine tetraacetic acid
LDL: low density lipoproteins (d=1.019 - 1.050 g/ml)
HDL: high density lipoproteins (d=1.075 - 1.21 g/ml)

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MATERIALS AND METHODS

Animals and cells: Adult male Wistar rats, about 200 g, were from Møllegård Hansens Avlslaboratorium, Copenhagen, Denmark. They were fed ad libitum with ordinary lab chow. Liver cell suspensions were prepared by a modification (11,12)of the method described by Berry and Friend (13). The non-parenchymal cells were prepared from liver cell suspensions treated with enterotoxin (14). A detailed description of the method of isolation of cells has been given previously (15).

Lipoproteins were obtained from rat EDTA plasma and fractionated by sequential flotation according to standard techniques using KBr for density adjustments (16). The lipoproteins were labelled according to the method of McFarlane (17) with the modification of Bilheimer et al. (18). In all preparations used more than 98% of the radioactivity was precipitable in 15% (w/v) trichloroacetic acid. Less than 3% (LDL) or 10% (HDL) of the radioactivity was extractable into chloroform/methanol. The lipoprotein preparation was free of albumin and lipoproteins from other density classes as tested by standard electrophoretic techniques.

Materials: Collagenase (type I), bovine serum albumin (fraction V), leupeptin were all from Sigma Chem. Co., St. Louis, Mo., USA. Dibutyl phthalate and dinonyl phthalate were from Koch Light Lab. Ltd., Colnbrook, U.K. Na(^{125}I) (carrier free) was from Institutt for Energiteknikk, Kjeller, Norway. Bovine serum albumin was treated by charcoal before use (19) and did not compete with the binding of labelled lipoprotein to the cells.

For the study of the interaction of labelled lipoproteins with the cells, the cells were suspended in 3-5 ml of the incubation medium and incubated in a shaking water bath. The cell concentration was $2 \cdot 10^6$ to $5 \cdot 10^6$ cells per ml. Following the addition of lipoprotein to the cell suspensions 200 µl samples were removed and the cells separated from the incubation medium by centrifugation through 250 µl dibutyl phthalate (hepatocytes) or a mixture (1:4) of dinonyl phthalate and dibutyl phthalate (non-parenchymal cells) in polypropylene microtubes as described earlier (6). Degradation was followed by measuring radioactivity soluble in trichloroacetic acid. 200 µl samples of the cell suspension were mixed with 400 µl of 30% (w/v) trichloroacetic acid, kept on ice for 30 min and centrifuged at 3000 rev./min for 10 min to separate acid soluble and acid precipitable radioactive material.

Subcellular fractionation: Hepatocytes were suspended in ice-cold 0.25 M sucrose solution and homogenized by about 25 strokes in a chilled Dounce homogenizer with a tight fitting pestle. Subcellular fractionation of the homogenate was done by isopycnic centrifugation: A postnuclear fraction of the homogenate was layered onto a linear sucrose gradient (density from 1.09 to 1.25 g/ml) in 36 ml centrifuge tubes. The tubes were centrifuged for 4 hrs at 25000 rev./min in a Beckman ultracentrifuge equipped with a SW-27 rotor. The gradients were fractionated into 2 ml fractions (20).

Biochemical determinations: Acid phosphatase (EC 3.1.3.2) was assayed as described by Barrett (21). 5'-Nucleotidase (EC 3.1.3.5) was assayed as described by El-Aaser & Reid (22). Protein was determined according to Lowry et al. (23) with bovine serum albumin as standard.

RESULTS AND DISCUSSION

The binding and uptake of (^{125}I)LDL to rat liver cells were initially rapid and then levelled of (Fig. 1a & 1b). At 4°C an apparent equilibrium between association and dissociation was reached after 2 hrs. At 37°C the cell-associated radioactivity increased slowly during the full length of the experiment (i.e. 3 hrs). If (^{125}I)HDL were added to hepatocytes the pattern of cell-association was similar to

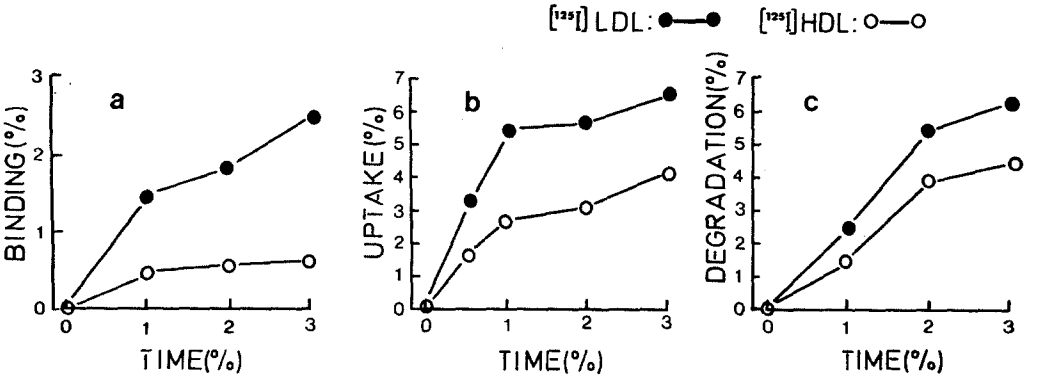

FIG. 1. Time courses of *binding* (a), *uptake* (b) and *degradation* (c) of (^{125}I)LDL and (^{125}I)HDL in isolated *hepatocytes*. The cells ($2 \cdot 10^6$ cells/ml) were incubated with 40 µg protein per ml of either (^{125}I)LDL or (^{125}I)HDL. Binding was measured at 4°C, uptake and degradation at 37°C. The results are expressed as per cent of available labelled lipoprotein in the medium.

that of (^{125}I)LDL (Figs. 1a & 1b). At 4°C, however, a higher percentage of (^{125}I)LDL than (^{125}I)HDL bound to the cells. The difference in uptake at 37°C was minor. The hepatocytes also degraded LDL and HDL at 37°C at a similar rate (Fig. 1c).

We then preincubated hepatocytes with increasing concentrations (50 nmol/l - 2 µmol/l) of unlabelled LDL and HDL for 1 h at 37°C. The concentrations given were based on

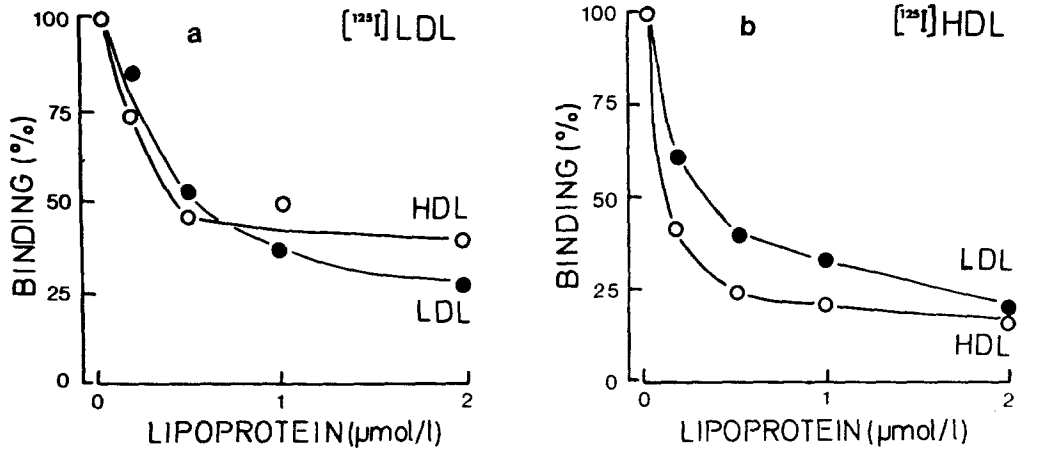

FIG. 2. *Binding* of (^{125}I)LDL (a) and (^{125}I)HDL (b) to rat *hepatocytes* in presence of increasing concentrations of unlabelled LDL and HDL. The cells ($2 \cdot 10^6$ cells/ml) were incubated with unlabelled lipoprotein for 1 h at 37°C. The cells were then reincubated at 4°C with either 50 nmol/l (^{125}I)LDL or (^{125}I)HDL for 2 hrs. Bound labelled lipoprotein is expressed as per cent of cell-associated radioactivity in cells incubated in absence of unlabelled lipoprotein.

mol. wts. of $2 \cdot 10^6$ daltons for LDL and $2 \cdot 10^5$ daltons for HDL (24). To the various cell suspensions we added 50 nmol/l (^{125}I)LDL (Fig. 2a) or (^{125}I)HDL (Fig. 2b) and continued the incubation at 4°C for 2 hrs. With increasing concentrations of unlabelled lipoprotein present during the preincubation a progressive reduction in binding of (^{125}I)LDL and (^{125}I)HDL was measured.

The addition of 3 mmol/l EDTA did not influence the binding of (^{125}I)LDL. Concanavalin A (0.1 mg/ml) increased cell bound (^{125}I)LDL ten-fold. Treatment of the cells with 0.25% pronase reduced the subsequent binding to about half of control values (results not shown).

To gain insight in the intracellular handling of (^{125}I)LDL in hepatocytes we fractionated homogenates from hepatocytes that had been exposed to (^{125}I)LDL *in vitro* and *in vivo*. The latter experiment was performed after having injected (^{125}I)LDL intravenously and prepared hepatocytes 3 hrs thereafter. Figs. 3a and 3b show the distribution of radioactivity, 5'-nucleotidase and acid phosphatase after isopycnic centrifugation in sucrose gradients. The distribution of (^{125}I)LDL injected intravenously followed the distribution of acid phosphatase (Fig. 3a). (^{125}I)LDL taken up *in vitro*, on the other hand, coincided with the distribution of 5'-nucleotidase (Fig. 3b).

Non-parenchymal liver cells were prepared by means of enterotoxins from *Clostridium perfringens* which damages the hepatocytes selectively (14). When (^{125}I)LDL and (^{125}I)HDL were incubated with the cells at 4°C we found that more LDL than HDL bound to the cells (Fig. 4a). If the experiment was performed at 37°C the difference between (^{125}I)LDL and (^{125}I)HDL was less striking (Fig. 4b). After 3 hrs of incubation at 4°C, the cells bound 0.6% and 3% of HDL and LDL, respectively (10 µg protein per ml). At 37°C we found that after a 30 min lag phase, the cells degraded both (^{125}I)LDL and (^{125}I)HDL at a linear rate. The rate of degradation corresponded to 2%/h of added lipoprotein for both types of lipoproteins (Fig. 4c).

To gain further insight in the intracellular degradation of LDL *in vitro*, both cell types were preincubated with 0.1 mg/ml of the thiol protease inhibitor

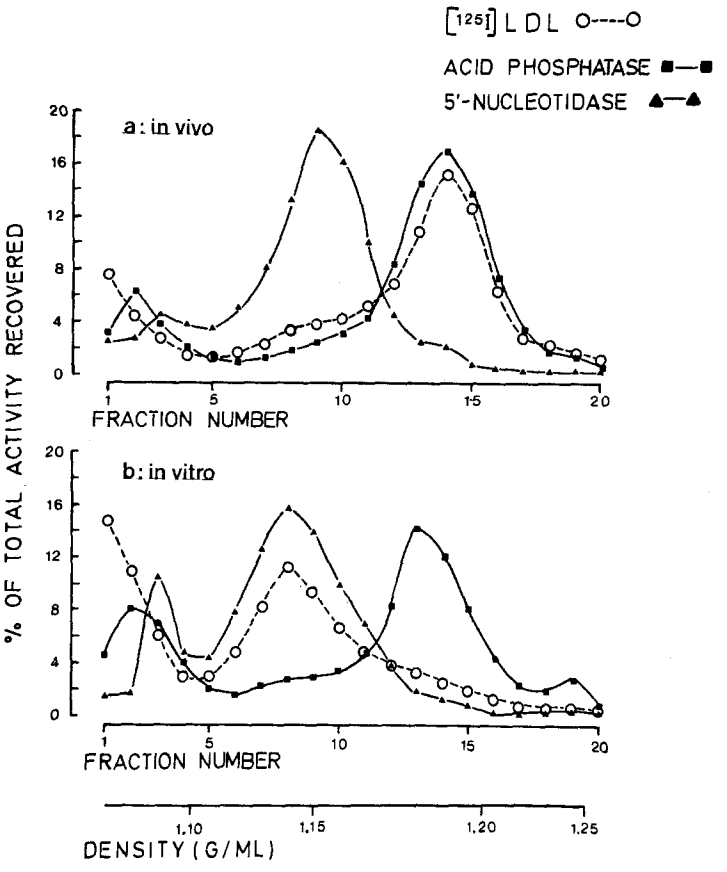

FIG. 3. *Distribution of (^{125}I)-radioactivity and marker enzymes* in sucrose gradients after isopycnic centrifugation of rat *hepatocyte* homogenates derived from cells exposed to (^{125}I)LDL *in vivo* (a) and *in vitro* (b). In the caudal vein of a rat was injected 300 µg labelled LDL and hepatocytes prepared 4 hrs later. In the *in vitro* (b) experiment the isolated hepatocytes ($5 \cdot 10^6$ cells/ml) were exposed to 80 µg/ml of (^{125}I)LDL for 2 hrs at 37°C. In both experiments the postnuclear fractions (from $20 \cdot 10^6$ cells) were layered onto sucrose gradients. The (^{125}I)-radioactivity and enzyme activities (acid phosphatase and 5'-nucleotidase) in the fractions are given as per cent of total recovered activity in the gradients. The fractions are presented in the order of their position in the centrifuge tube from the meniscus (left) to the bottom (right).

leupeptin for 30 min before (^{125}I)LDL were added. Uptake and degradation of (^{125}I)LDL were determined simultaneously (Figs. 5a-5d). The leupeptin-treated cells accumulated slightly more of the labelled LDL (Figs. 5a & 5c) and they also degraded less of the lipoproteins (Figs. 5b & 5d). The measured degradation represents the cell-dependent degradation as the degradation in the incubation medium was subtracted (6).

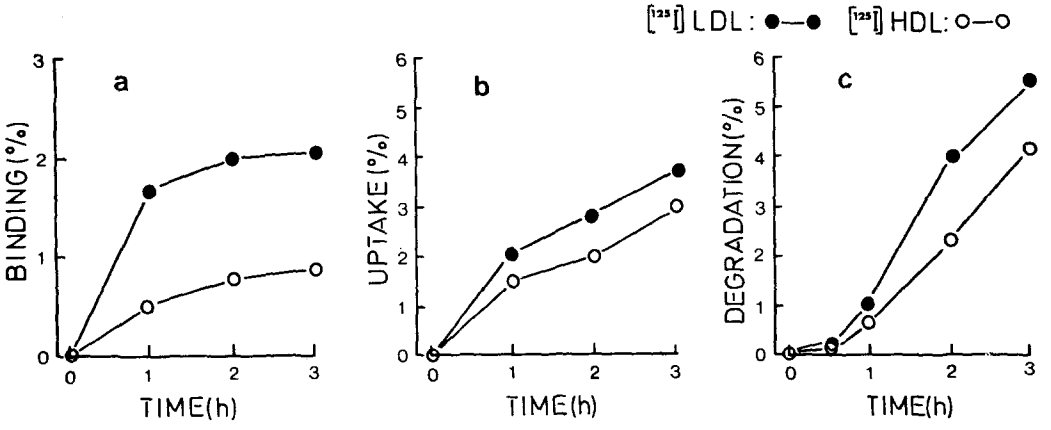

FIG. 4. Time courses of *binding* (a), *uptake* (b) and *degradation* (c) of (^{125}I)LDL and (^{125}I)HDL to isolated rat *non-parenchymal liver cells*. The cells ($5 \cdot 10^6$ cells/ml) were incubated with 12.5 µg protein per ml of either (^{125}I)LDL or (^{125}I)HDL. Binding was measured at 4°C and the uptake and degradation at 37°C. The results are expressed as per cent of available lipoprotein in the medium.

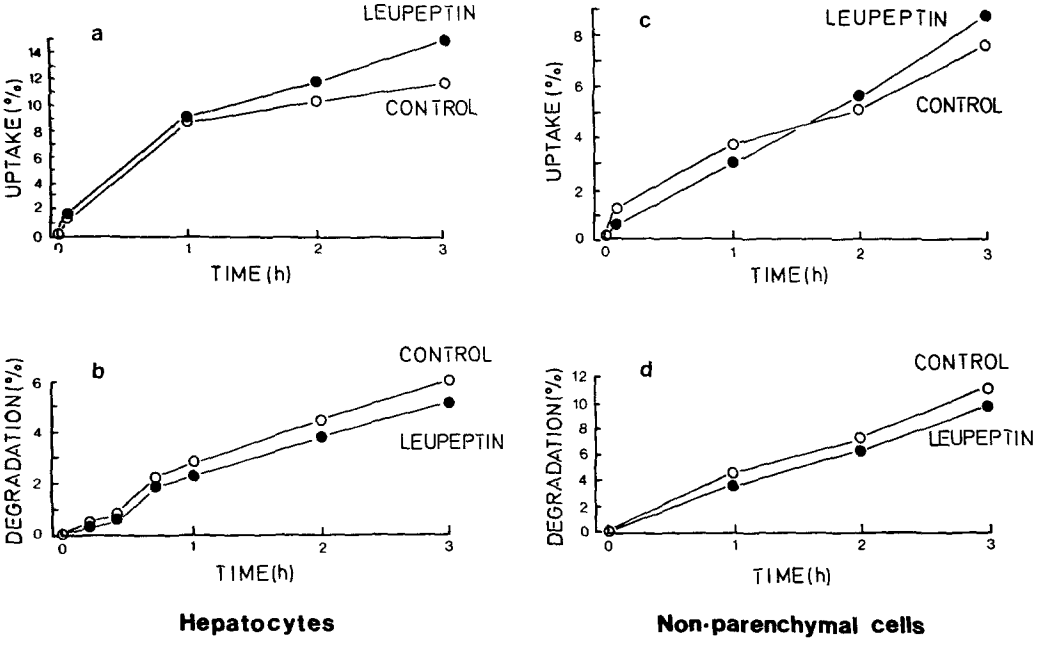

FIG. 5. The effect of *leupeptin* on the *uptake* (a & c) and *degradation* (b & d) of (^{125}I)LDL in *hepatocytes* (a & b) and *non-parenchymal liver cells* (c & d). The cells were preincubated for 30 min at 37°C with 0.1 mg/ml leupeptin. To the hepatocytes ($9 \cdot 10^6$ cells/ml) and the non-parenchymal cells ($7 \cdot 10^6$ cells/ml) were added 30 µg/ml of labelled LDL. The results are expressed as per cent of available lipoprotein in the medium.

This report shows that both rat hepatocytes and non-parenchymal liver cells bind (^{125}I)LDL in addition to (^{125}I)HDL (6). The binding site is not specific exclusively for LDL. The binding does not require Ca^{++} in contrast to the LDL receptor in fibroblasts (10) and the asialo-glycoprotein receptor in hepatocytes (25).

The LDL uptake in non-parenchymal cells was relatively efficient, but could evidently not be explained by the presence of the specific LDL receptor. About 35% of the non-parenchymal cells prepared by the enterotoxin method are macrophages (Kupffer cells) (14). There is no evidence from studies on other types of macrophages that such cells possess specific LDL receptors (26).

The rate of LDL degradation far exceeds what can be attributed to fluid endocytosis in these cells: i.e. 0.08 µl/h/10^6 cells for hepatocytes and 0.07 µl/h/10^6 cells for non-parenchymal cells (27).

The total LDL degradation would require an endocytic uptake of fluid by the cells which is 30 times and 75 times higher in hepatocytes and non-parenchymal cells, respectively, than the rates observed for fluid endocytosis in these cells. The effect of leupeptin was about similar in hepatocytes and non-parenchymal liver cells. Only about 15-20% of the degradation in the liver cells can be attributed to thiol proteases in the lysosomes. Even this pathway degraded about 6-10 times more LDL than could be attributed to fluid endocytosis.

In order to get information about the intracellular distribution of (^{125}I)LDL isopycnic centrifugation was applied. The results indicated that *in vivo* an accumulation of (^{125}I)LDL takes place in the lysosomes. (^{125}I)LDL taken up by the cells *in vitro* accumulated in organelles coinciding with the plasma membrane marker 5'-nucleotidase in sucrose gradients. These results are identical to data published previously on the distribution of cell-accumulated (^{125}I)HDL in hepatocytes (28). We proposed that HDL accumulated *in vitro* both on the plasma cell membrane and in an endocytic vesicle with density about 1.13 g/ml in sucrose gradients. In conclusion, we propose that both liver cell types possess a

route for internalization and degradation of (^{125}I) LDL. This pathway is different from that mediated by the specific LDL receptor(10). The process of internalization is preceded by binding to the cell membrane.

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