THE EFFECT OF SELECTIVE MODIFICATION OF THE ARGINYL RESIDUES OF PARTIALLY-METABOLIZED VERY LOW DENSITY LIPOPROTEINS ON THEIR UPTAKE BY THE LIVER

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1. Introduction

The metabolism of plasma chylomicrons and VLDL by LPL of the extrahepatic tissues results in the depletion of their core triacylglycerol and the loss of a proportion of their surface protein, phospholipid and cholesterol to the plasma HDL [1,2]. In the rat the resultant modified lipoproteins are avidly removed from the circulation by the liver and this process of removal is saturable and receptor-mediated [3,4].

The receptor-mediated binding and uptake of plasma LDL by fibroblasts is inhibited by the specific reaction of the guanido groups of the arginine residues of the lipoproteins with 1,2-cyclohexanedione [5]. Regeneration of the groups by treatment with hydroxylamine re-establishes binding and uptake. Here, we report that cyclohexanedione treatment similarly reversibly inhibits the uptake of partially-metabolized VLDL by the perfused rat liver.

2. Materials and methods

2.1. *Animals*

Male Wistar albino rats were used and their source, maintenance diet and weight range were as specified in [4].

2.2. *The production of partially-metabolized VLDL*

Nascent VLDL, labelled in their triacylglycerol fatty acids with [^{14}C]oleic acid, were isolated from rat liver perfusates as in [4]. Functionally hepatectomized and eviscerated rats [3] were each injected via the left jugular vein with 0.4–0.5 ml nascent VLDL (containing 13 mg triacylglycerol/ml). The animals were bled into tubes containing EDTA (final conc. 1 mg/ml) by cannulation of the abdominal aorta 40 min after the injection. Partially-metabolized plasma VLDL were isolated as in [4] after the removal of erythrocytes by centrifugation.

2.3. *1,2-Cyclohexanedione modification of partially-metabolized VLDL apoproteins*

Conditions for the modification of arginyl residues of proteins by 1,2-cyclohexanedione have been described [5,6]. Here, an aliquot (2 ml) of partially-metabolized VLDL containing ~2 mg protein/ml lipoprotein solution was mixed with 4 ml of a solution of 1,2-cyclohexanedione (0.15 M) in borate buffer (0.2 M) (pH 8.1) and incubated at 35°C for 2 h. The sample was then dialysed for 24 h against 0.9% NaCl solution and kept at 4°C until required.

Modification of the arginyl residues by 1,2-cyclohexanedione is a reversible process. Native residues were regenerated by incubation of the dialysed 1,2-cyclohexanedione-treated lipoprotein with an equal volume of hydroxylamine (1 M) in mannitol (0.3 M) (pH 7.0) at 37°C for either 7 or 16 h [5,6]. The lipoprotein solution was then exhaustively dialysed against 0.9% NaCl solution.

2.4. *Liver perfusions for studies on the removal of partially-metabolized VLDL*

A recycling liver perfusion system [4] was used to study the removal of untreated, 1,2-cyclohexanedione-treated and hydroxylamine-treated partially-

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LPL, lipoprotein lipase

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metabolized VLDL. In one group of experiments, the removal of untreated and 1,2-cyclohexanedione-treated lipoprotein was compared while in another the removal of untreated, 1,2-cyclohexanedione-treated and hydroxylamine-treated lipoprotein was examined. For each comparison, a single batch of partially-metabolized VLDL obtained from 4 perfused livers was used and 18 functionally hepatectomized and eviscerated rats were injected for the preparation of partially-metabolized VLDL.

After the addition of lipoprotein to the perfusion medium, the uptake of [^{14}C]triacylglycerol-labelled lipoprotein by the perfused liver was monitored by following the removal of radioactivity at intervals up to 25 min. At each time interval, 0.5 ml perfusion medium was removed and, after centrifugation to remove erythrocytes, 130 μl supernatant was used for radioactivity measurements.

2.5. *Analytical methods*

Triacylglycerol, protein and radioactivity were measured as in [2]. For amino acid analysis, the delipidated apoprotein [7] was hydrolysed in HCl (6 M) for 24 h at 110°C [5] and the amino acid composition determined by Dr J. B. C. Findlay of the Department of Biochemistry, University of Leeds, by analysis on an automated analyzer, Rank-Hilger Chromaspek J180. The peak areas were integrated by a Digico Micro 16 V computer.

Electron microscopy was carried out by Mr D. Kershaw at the Department of Biochemistry, University of Leeds.

3. Results

Changes in the lipid and protein composition of VLDL following their metabolism by the extrahepatic tissues of the rat have been determined in [2,8]. Here, the metabolism of injected VLDL in the hepatectomized rat resulted in the loss of some 60% of the injected triacylglycerol radioactivity by 40 min.

In [5] 1,2-cyclohexanedione reacted with exposed arginyl guanido groups of lipoproteins and quantitative regeneration of the arginyl residues was achieved by incubation of the 1,2-cyclohexanedione-treated lipoprotein with hydroxylamine. The data in table 1 show that treatment with 1,2-cyclohexanedione under these conditions blocks some 50% of the arginyl residues of partially-metabolized VLDL and that

Table 1
Amino acid composition of partially-metabolized rat VLDL apoprotein (no. residues/mol apoprotein)

Amino acid	Untreated VLDL	1,2-Cyclohexane-dione-treated VLDL	1,2-Cyclohexane-dione-treated and hydroxylamine-regenerated VLDL
Asx	23	20	20
Thr	22	25	22
Ser	21	21	19
Glx	38	30	30
Pro	12	15	12
Gly	19	21	19
Ala	18	19	17
Val	16	17	15
Met	7	5	6
Ile	9	11	10
Leu	30	28	27
Tyr	7	6	6
Phe	10	8	8
Lys	17	15	17
Arg	12	6	12

VLDL was delipidated with ethanol:ether; 1 mg apoprotein was hydrolyzed with HCl (6 M) in the presence of 30 μl of mercaptoacetic acid at 110°C for 24 h in a sealed ampoule flushed with nitrogen. Amino acid residues are calculated assuming a total of 250 amino acid residues/mol of VLDL apoprotein [5]

these are regenerated on incubation of the lipoprotein preparation with hydroxylamine. The percentage of arginyl residues modified is very similar to that in [5].

Negative-staining electron microscopy of the untreated and the 1,2-cyclohexanedione-treated lipoproteins showed no difference in size or morphological appearance. The size of the particles ranged from 150–1000 Å, with some 85% of the particles being the range of 300–600 Å.

3.1. *Removal of untreated, 1,2-cyclohexanedione-treated and hydroxylamine-regenerated, partially-metabolized VLDL by the perfused liver*

The triacylglycerol-rich lipoprotein fraction isolated from the plasma of the functionally hepatectomized and eviscerated rats contained partially-metabolized lipoproteins originating from the injected ^{14}C-labelled VLDL and from endogenous chylomicrons and VLDL. The initial concentration of these lipoproteins (^{14}C-labelled injected and endogenous) in this perfusion medium was 130–300 μg triacylglycerol/ml. This range was below that required to saturate the receptor-mediated uptake mechanism (see [4]).

Partially-metabolized VLDL are taken up by the perfused rat liver by a process that involves the uptake of the intact lipoprotein and the process can, therefore, be followed by monitoring the removal of the ^{14}C-labelled triacylglycerol moiety of the perfused lipoprotein [4]. Fig.1 compares the removal of the ^{14}C-labelled triacylglycerol of untreated, 1,2-cyclohexanedione-treated and hydroxylamine-regenerated, partially-metabolized VLDL by the perfused liver. Over 25 min, some 60% of the untreated lipoprotein is removed (fig.1a). Modification of the arginyl residues by 1,2-cyclohexanedione virtually abolishes this removal (fig.1b). When the arginyl residues are regenerated by hydroxylamine treatment, removal again occurs and the extent of the removal increases with the time of exposure to hydroxylamine (fig.1c,d).

4. Discussion

The surface features of partially-metabolized chylomicrons and VLDL that are recognized by receptors in the liver of the rat have not been fully elucidated. The loss of surface apoprotein C from the parent lipoproteins during triacylglycerol hydrolysis by LPL may be a prerequisite [9,10], as may be the loss of

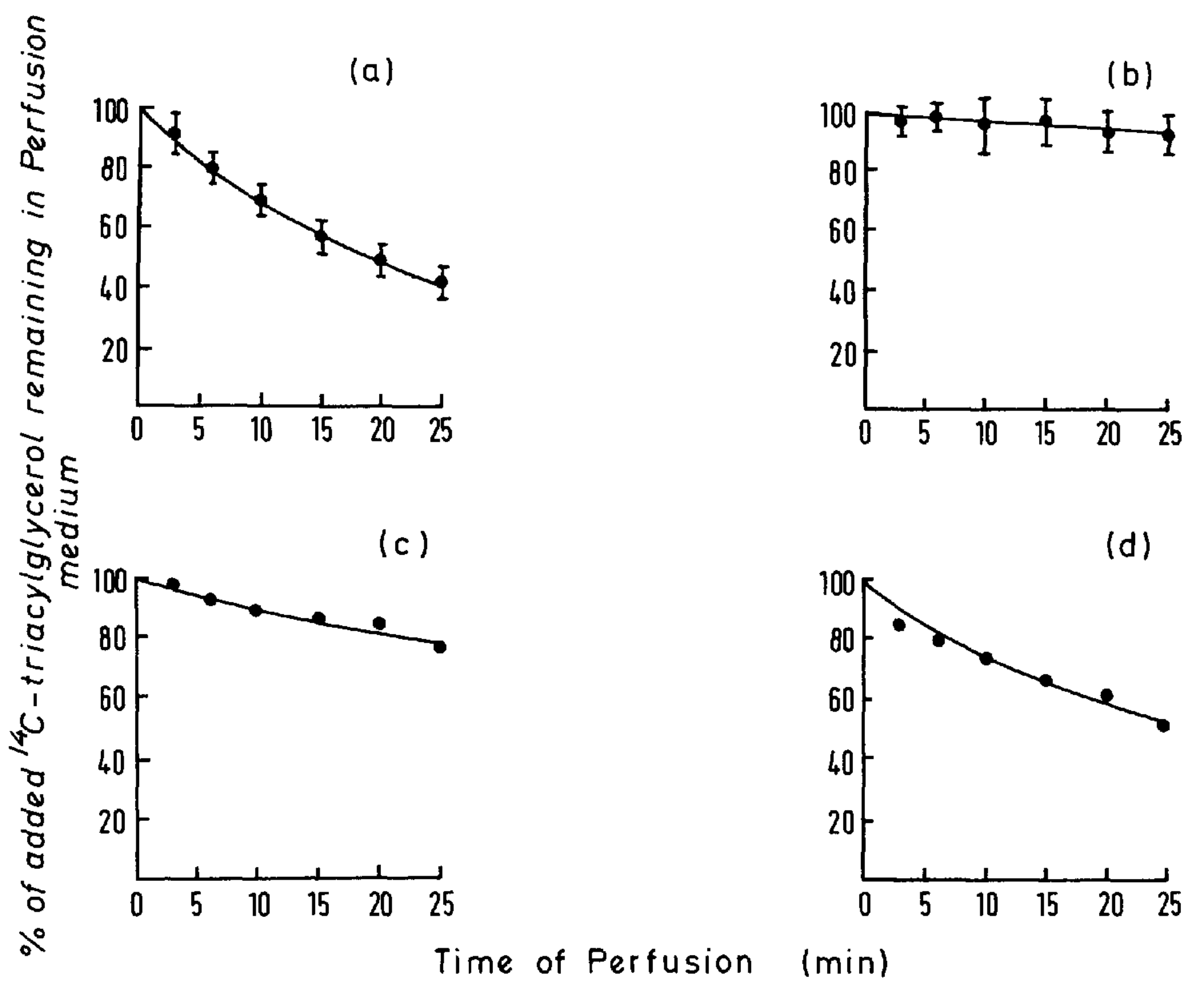

Fig.1. Removal of partially-metabolized VLDL by the perfused liver: (a,b) removal of untreated and 1,2-cyclohexanedione-treated, partially-metabolized VLDL, respectively; (c,d) removal of 1,2-cyclohexanedione-treated particles that had been incubated with hydroxylamine for 7 h and 16 h, respectively. (a,b) mean (±SD) removal measured during perfusions of 6 livers; (c,d) mean removal measured during 2 liver perfusions.

surface phospholipid [11] and a decline in size of the lipoproteins [12]. However, a rise in the proportion of apoprotein E on the surface of the partially-metabolized lipoprotein may well be of major importance. For example, apoprotein E addition promotes the hepatic uptake of chylomicrons [9,10] and HDL_c, a lipoprotein containing predominantly apoprotein E, competes with partially-metabolized chylomicrons for the same hepatic receptor-binding sites [13].

On the basis of the foregoing, it would be reasonable to assume that it is the reaction of 1,2-cyclohexanedione with the arginine residues of the apoprotein E component of the partially-metabolized VLDL that is responsible for the inhibition of removal of these lipoproteins by the perfused liver here. However, partially-metabolized VLDL contain a number of apoprotein species, notably apoprotein B, and 1,2-cyclohexanedione can presumably react with the arginine residues of several of these. Moreover, [14,15] suggest that the hepatic receptor can recognize apoprotein B as well as apoprotein E. It is therefore certainly not possible to exlude the participation of other apoproteins in the removal process.

The receptor-mediated binding and uptake of the plasma LDL by fibroblasts involves interaction with the apoprotein B component of the lipoprotein. However, this same receptor also binds, and with higher affinity, the apoprotein E of HDL_c [16] and in addition binds partially-metabolized chylomicrons that contain both apoproteins B and E [17]. On the other hand, the liver receptor concerned with the uptake of partially-metabolized chylomicrons and VLDL has been shown under appropriate conditions to also bind both HDL_c and LDL [18]. These observations have led us to the suggestion that the hepatic and extra-hepatic high affinity lipoprotein receptors resemble each other closely and that both receptors recognize similar peptide sequences in the apoproteins [18]. The present observation that the blockage of arginine residues with 1,2-cyclohexanedione prevents the uptake of partially-metabolized VLDL by rat liver, as it does the uptake of LDL and HDL_c by fibroblasts [5], lends further support to this view. The report [19] showing that the Watanabe-heritable hyperlipidaemic rabbit lacks both the LDL receptor and the hepatic receptor for partially-metabolized chylomicrons, suggests indeed a common genetic origin for the two receptors.

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