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VLDL apoprotein secretion and apo-B mRNA level in primary culture of cholesterol-loaded rabbit hepatocytes

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Incubation of cultured rabbit hepatocytes with β very low density lipoproteins (β-VLDL) induces a dose-dependent increase in cell cholesterol (CH) content and VLDL apoprotein (apo) B and E secretion without change in apo-B mRNA level. These data suggest that β-VLDL may exert a stimulatory effect on hepatic apo-B production at the co-translational and/or posttranslational level.

β-VLDL; Cholesterol; VLDL secretion; Apolipoprotein B; mRNA; (Rabbit hepatocyte)

1. INTRODUCTION

Feeding rabbits a CH-rich diet induces high hypercholesterolemia, accumulation of cholesterol ester-rich VLDL in plasma and significant increase in hepatic CH content [1,2]. Accumulation of hepatic CH causes inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase (EC 1.1.1.34) activity, suppression of CH synthesis and a profound decrease in the production of LDL receptors [3].

Recent studies have shown that CH diet also increases the rate of apo-E synthesis in rabbit liver [4] and VLDL protein secretion by perfused liver [5]. The stimulation of VLDL apo synthesis by hepatic CH overload was confirmed in experiments on isolated rat liver perfused with CH-rich lipoproteins [6]. At the same time, Kroon et al. [7] demonstrated that livers of CH-fed rabbits have lower apo-B mRNA levels compared to control animals. From these data the authors suggested that the accumulation of plasma β-VLDL in CH-fed rabbits is not due to increased rates of its production. The explanation for these discrepancies is unclear and requires further investigation.

Here, we measured the rate of VLDL apo secretion and cell apo-B mRNA level after CH loading of rabbit hepatocytes on incubation with β-VLDL in primary culture.

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Abbreviations: β-VLDL, beta very low density lipoproteins; apo, apoprotein; CH, cholesterol; ECH, cholesterol esters

2. MATERIALS AND METHODS

2.1. *Materials*

All chemicals used were reagent grade. Minimal essential medium with Earle's salts (ME medium), fetal calf serum (FCS), MEM non-essential amino acids, kanamycin, L-glutamine and culture dishes were purchased from Flow Laboratories. Collagenase type IV was a product of Sigma (St. Louis, MO). L-[^{35}S]Methionine (spec. act. >800 Ci/mmol), [1-^{14}C]acetic acid, sodium salt (spec. act. 59 mCi/mmol) and L-[^{32}P]dATP (>1000 Ci/mmol) were purchased from Amersham International (Amersham, England).

2.2. *Preparation of lipoproteins*

Hyperlipemic β-VLDL was isolated from rabbits receiving a suspension of CH in olive oil (0.2 g CH/kg body wt per os) each day for 3 months. Plasma CH levels ranged from 800 to 1100 mg/dl. The β-VLDL (d < 1.006 g/ml) fraction was isolated by centrifugation at 45000 rpm for 18 h at 4°C and

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washed by recentrifugation in 0.15 M NaCl at 45000 rpm for 18 h [8]. The average mass ratio of CH to protein in rabbit β-VLDL ranged from 5.2 to 6.1. The apo content was determined by SDS-polyacrylamide gel electrophoresis. β-VLDL contained predominantly apo-E, apo-B100+48 and small quantities of apo-Cs.

2.3. *Cultured hepatocytes*

Rabbit hepatocytes were prepared and plated after collagenase digestion as described in [9]. Cells were plated in plastic dishes at 2×10^5 cells/cm^2 in ME medium containing 10% FCS, kanamycin (100 μg/ml) and non-essential amino acids (1 mM) and were maintained at 38°C in a 95% air-5% CO_2 atmosphere. Cells were incubated for 24 h under these conditions. After another 24 h in ME medium containing 10% lipoprotein-deficient serum (LDS) with and without different concentrations of β-VLDL, cells were used for metabolic studies.

2.4. *Metabolic studies*

Isotope labeling studies, VLDL isolation and analysis, determination of the secretion rates of VLDL ^{35}S-labeled apos and ^{14}C-labeled lipids and lipid analysis were carried out as described by us previously [10]. Protein concentration was determined by the method of Lowry et al. [11] using bovine albumin as a standard.

The partial rabbit apo-B gene clone has been described elsewhere [12]. Total RNA was isolated from hepatocytes using the guanidine isothiocyanate procedure [13]. All manipulations with RNA and DNA were performed mainly as in [14].

3. RESULTS

Quantitative analysis of the intracellular lipids has shown that free CH and cholesterol esters (ECH) content were significantly increased in cells incubated with β-VLDL (table 1).

Table 1

Effect of β-VLDL on CH content of rabbit hepatocytes

β-VLDL added (μg/ml β-VLDL)	CH (μg/ml cell protein)	ECH
Expt 1		
None	27.4 ± 3.6	8.4 ± 1.6
+25	47.2 ± 4.8[a]	28.2 ± 3.1[a]
+100	68.6 ± 4.8[b]	72.2 ± 9.4[b]
Expt 2		
None	32.2 ± 3.9	6.2 ± 1.0
+25	43.1 ± 3.5[a]	21.3 ± 3.0[a]
+100	60.4 ± 7.0[b]	42.2 ± 5.0[b]

Results represent the means ± SD of three replicates. Statistical significance from control: [a] $p < 0.05$; from another experiment [b] $p < 0.05$

To define the effect of β-VLDL preincubation with cells on the secretion of newly synthesised VLDL apos, cells were cultured with 100 μg/ml β-VLDL for 24 h. After incubation the medium was replaced by fresh medium, containing [^{35}S]methionine and secreted VLDL apoproteins were analysed. Fig.1 shows the autoradiogram of SDS-polyacrylamide gel electrophoresis and the stimulation of [^{35}S]methionine incorporation into apo-B,E and C after cultivation of the control, and CH-loaded cells for 18 h. Table 2 lists the amounts of radioactivity recovered in the VLDL apo bands. β-VLDL produced a 2–8-fold increase in the amount of apo-B and apo-E secreted by hepatocytes after 18 h cultivation. The amount of VLDL total apo and labeled apo-B increased with increasing doses of β-VLDL after 8 h cultivation (fig.2).

To estimate the level of hepatocyte apo-B mRNA we used RNA blot hybridization analysis utilising a rabbit genomic apo-B probe [12]. Fig.3

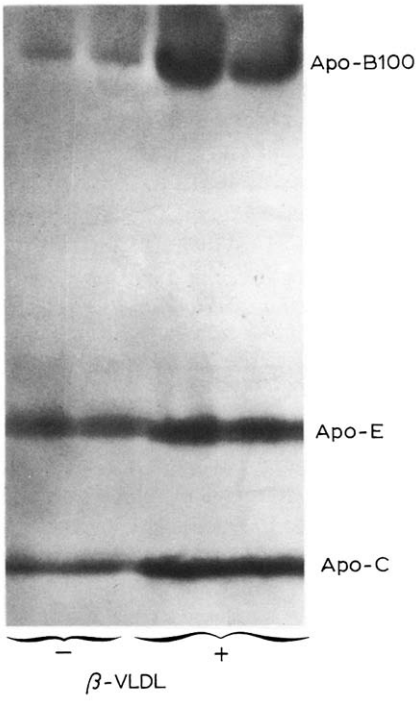

Fig.1. Autoradiogram of newly synthesized and secreted VLDL apos. The gel (3–20% gradient polyacrylamide) was loaded with the VLDL fraction derived from an equal volume of medium (5 ml). This would correspond to the VLDL secreted by 4.0 ± 0.3 mg cell protein.

Table 2

Effect of β-VLDL on the rates of ^{35}S-labeled VLDL apo secretion

β-VLDL added	Apo-B (10^{-3} cpm/mg cell protein)	Apo-E
Expt 1		
None	18.0 ± 1.6	29.7 ± 6.7
β-VLDL	141.2 ± 17.7[a]	86.0 ± 2.1[a]
Expt 2		
None	24.4 ± 3.6	16.8 ± 1.9
β-VLDL	41.1 ± 5.9[a]	51.1 ± 3.1[a]
Expt 3		
None	32.2 ± 3.3	–
β-VLDL	97.3 ± 11.9[a]	–

Results represent 6 means ± SD of three replicates. Statistical significance from control: [a] $p < 0.05$

depicts dot blots of total RNA from control and CH-loaded rabbit hepatocytes. After autoradiography the dot blots were quantitated by laser densitometry. After 24 h incubation with 100 μg/ml β-VLDL the level of apo-B mRNA was unaltered compared to control cells. Similar results were obtained in three experiments.

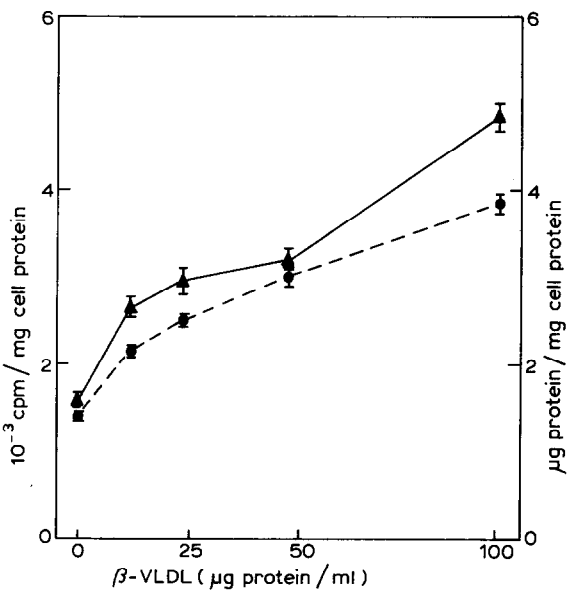

Fig.2. Effect of prior incubation with β-VLDL on VLDL ^{35}S-labeled apo-B (▲—▲) and VLDL total protein (●---●) secretion by rabbit hepatocytes.

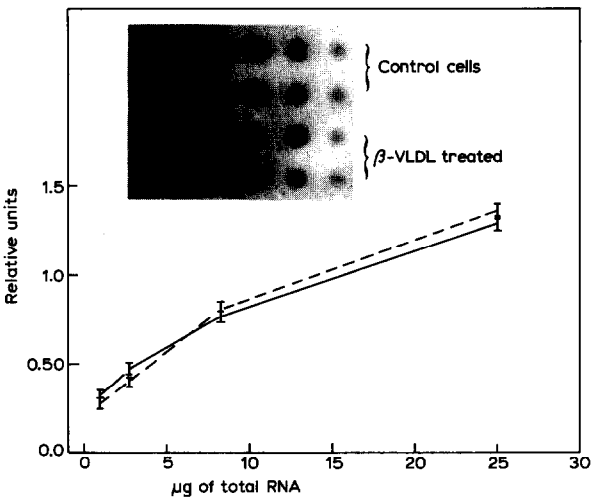

Fig.3. Modulation of rabbit hepatic apo-B mRNA levels by β-VLDL. Autoradiograms of the dot blot of total RNA isolated from rabbit hepatocytes cultured in ME medium (10% LDS) with (lane 1) and without (lane 2) 100 μg/ml β-VLDL after hybridization with ^{32}P-labeled apo-B probe. Densitometry of the autoradiogram is shown below. Results represent means ± SD of three replicates.

4. DISCUSSION

The present data demonstrate that VLDL apo secretion from cultured rabbit hepatocytes is stimulated after incubation with β-VLDL. The increase is dose-dependent and the degree of intracellular CH loading correlates with the amount of VLDL apo secretion. These data confirmed our earlier finding [15], according to which free CH added in primary culture in ethanol solution can stimulate secretion of apo-B VLDL. Recently it was found [6] that hepatic CH overload stimulates the secretion of VLDL apos isolated from rat liver perfused with CH-rich lipoproteins. From these observations it can be suggested that intracellular CH accumulation increases hepatic secretion of VLDL apos.

To determine whether the increased accumulation of secreted apo-B is realized at the mRNA level or is attributable to an effect of β-VLDL on another stage of apo-B synthesis and/or secretion we determined the apo-B mRNA level. The data obtained show that the level of apo-B mRNA in CH-loaded hepatocytes is unaltered compared to that in control cells. These data suggest that β-

VLDL exerts its effect on apo-B production at the co-translational or posttranslational level.

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