HEPATIC ENDOTHELIAL LIPASE ANTISERUM INFLUENCES RAT PLASMA LOW AND HIGH DENSITY LIPOPROTEINS IN VIVO

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1. Introduction

Postheparin plasma contains two separate lipolytic enzymes, lipoprotein lipase and hepatic lipase, which are released from vascular endothelial cells. The former is located in extrahepatic capillary beds and has a well-defined role in the catabolism of plasma triglycerides. The hepatic lipase has been recently shown to be located on the surface of hepatic endothelial cells [1] but the physiological function of this enzyme is still far from clear. Hepatic endothelial lipase can hydrolyze chylomicron and VLDL triglycerides in vitro [2] but its activity in postheparin plasma has no correlation to plasma triglyceride levels [3]. It has been suggested that hepatic lipase could be involved in the uptake of chylomicron remnants [4], intermediate density lipoprotein [5] or LDL [6] by the liver but there is little experimental evidence for any of these possibilities.

We have recently purified the heparin-releasable lipase from rat liver perfusates [7] and used the enzyme preparation for production of antiserum. The availability of this anti-hepatic lipase serum offered a good opportunity to study the function of the enzyme by searching whether plasma lipoproteins are influenced by specific inhibition of the hepatic endothelial lipase in vivo.

Abbreviations: HDL, high density lipoprotein; HEL, hepatic endothelial lipase (previous term 'hepatic lipase' has not been used for the heparin-releasable lipase because the liver contains a number of other lipolytic enzymes); LDL, low density lipoprotein; VLDL, very low density lipoprotein

2. Methods

2.1. *Animals*

Male Sprague-Dawley rats (300–400 g) were used. Polyethylene catheters (PE-50, Clay Adams, USA) were inserted into jugular vein and carotid artery under phenobarbital anesthesia (Nembutal® 25 mg/kg body wt) and the animals were allowed to regain full consciousness over 2–3 h. During the experiment the rats were immobilized into small cages and they received water ad libitum but no food. The injections were given through the venous cannula while blood was sampled through the arterial catheter. Adult white New Zealand rabbits were used for production of the antiserum.

2.2. *Preparation of anti-hepatic lipase serum*

Heparin-releasable hepatic lipase was purified from rat liver heparin perfusate by chromatography on heparin–Sepharose column followed by ammonium sulphate precipitation and gel filtration on Ultrogel AcA 34 as described [7]. The purified enzyme exhibited a single major band in polyacrylamide gel electrophoresis and its specific triglyceride lipase activity was 5 mmol FFA $.h^{-1}$ $.mg^{-1}$ which is 54-times higher than that of the original perfusate. For production of antiserum 5–10 μg enzyme protein was mixed with equal volume of complete Freund's adjuvant and injected subcutaneously into rabbits at 4 week intervals. After 3–4 immunizations the antiserum showed high inhibitory titres against rat postheparin plasma hepatic lipase without having any effect on the lipoprotein lipase activity of the same plasma. A control serum was

obtained from normal nonimmunized rabbits. Lipoproteins were removed from both anti-HEL serum and control rabbit serum before their use for the experiments. The sera were mixed with NaCl–KBr solution to produce a solvent density of 1.21 g/ml and centrifuged in Sorvall OTD-2 ultracentrifuge with 42 000 rev./min for 40 h. The bottom layer was separated and extensively dialyzed against 0.15 M NaCl at +4°C. The anti-hepatic lipase activity of the antiserum was completely recovered in this fraction.

2.3. *Assay of lipase activities*

The assay of triglyceride lipase activity was based on measurement of the release of labeled free oleic acid from a gum arabic stabilized sonicated emulsion of triolein and tri[1-^{14}C]oleoylglycerol. Details of the preparation of substrate, incubation conditions and separation of free oleic acid from unhydrolyzed substrate were exactly as described [8]. In post-heparin plasma the hepatic lipase was defined as the activity present at 1.0 M NaCl without addition of serum; respectively, the lipoprotein lipase was taken as the activity measured at 0.15 M NaCl after incubation of the sample with anti-hepatic lipase serum [8].

2.4. *Preparation of postheparin plasma*

Non-fasting conscious rats were given an intravenous bolus of 500 IU heparin/kg body wt. After 2 min, 1 ml blood was withdrawn through arterial cannula, plasma was separated and used for lipase assays within the same day.

2.5. *Chemical methods*

Serum was separated by centrifugation at +4°C and stored at this temperature. Lipoproteins were isolated by sequential ultracentrifugation at solvent densities of 1.006 and 1.063 g/ml as in [9]. A Sorvall OTD-2 ultracentrifuge and Beckman Ti-50 angle head rotor were used. VLDL was separated at 1.006 g/ml by ultracentrifugation with 42 000 rev./min at +2°C for 18 h. The density of the infranate was adjusted to 1.063 g/ml by NaCl–KBr solution and the LDL was brought to the surface by ultracentrifugation for 20 h. The 1.063 infranate was taken to represent HDL. The recovery of each lipid in the three lipoprotein fractions varied from 93–100% of the corresponding total serum value. A correction for recovery was made.

Cholesterol was determined by an enzymatic method [10] using a commercial kit (Boehringer Mannheim). Phospholipids were measured according to [11], triglycerides by a Technicon Autoanalyzer [12] and protein by the Lowry method [13].

2.6. *Statistical analyses*

All values are given as means ± SEM. The significance of differences was calculated using the two-tailed Student's *t*-test.

3. Results

3.1. *Effect of anti-hepatic lipase serum injection on postheparin plasma lipolytic activities*

To determine the degree, duration and specificity of inhibition of hepatic lipase by the antiserum in vivo unanesthetized rats were given 1.5 ml of the lipoprotein-free anti-HEL serum or control serum as a single intravenous injection. Postheparin plasma was then obtained at various time intervals, once from each animal. The amount of antiserum used per animal was able to completely inhibit the hepatic triglyceride lipase activity of 42 ml rat postheparin plasma in vitro. After injection of the antiserum no

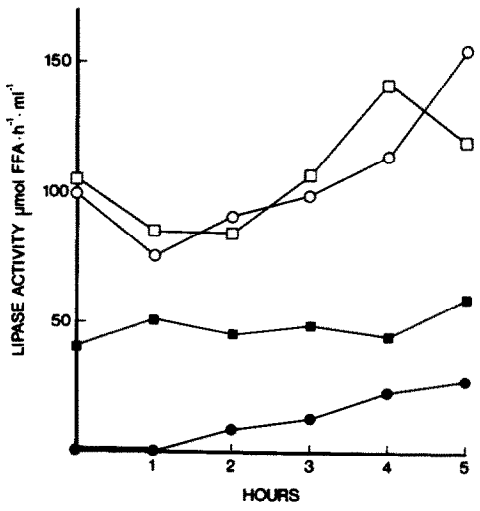

Fig.1. Postheparin plasma lipoprotein lipase (open circles) and hepatic lipase (black circles) activities after intravenous injection of anti-hepatic lipase serum (circles) or normal rabbit serum (squares) 5 min before the zero time. Each point represents a mean value obtained from 3 rats.

hepatic lipase activity could be detected in post-heparin plasma at 1 h whereafter the activity rose progressively, being 20% and 70% of the corresponding control value at 2 h and 5 h, respectively (fig.1). No significant change occurred in the lipoprotein lipase activity of postheparin plasma.

3.2. *Effect of anti-hepatic lipase serum on lipoproteins*

Ten unanesthetized rats with indwelling venous and arterial catheters were given 2 intravenous injections of lipoprotein-free anti-HEL serum (each dose neutralizing hepatic lipase in 42 ml postheparin plasma) at 2.5 h interval. Another 10 rats served as controls and received similar volumes of lipoprotein-free normal rabbit serum. The concentrations of cholesterol, phospholipids and triglycerides in the 3 major serum lipoprotein fractions were determined in samples drawn 5 h after the first antiserum injection. The results appear in fig.2. The animals injected with the antiserum had significantly higher concentrations of LDL and HDL cholesterol and phospholipids than the control rats. The mean total cholesterol level was 49% higher (105 mg/100 ml versus 70 mg/100 ml, $p < 0.001$) and the mean total phospholipid 28% higher (188 ± 7 mg/100 ml versus 147 ± 5 mg/100 ml, $p < 0.001$) in the antiserum group than in the control group. The triglyceride levels in whole serum and in all lipoprotein fractions were similar in the two groups. The cholesterol/phospholipid ratio in whole serum and in the lipoproteins was slightly higher in antiserum-treated rats than in the controls but the differences were not significant at 5% level. The protein concentration was determined only in LDL and it was similar in antiserum (33.0 ± 2.4 mg/100 ml) and control (30.5 ± 3.2 mg/100 ml) animals.

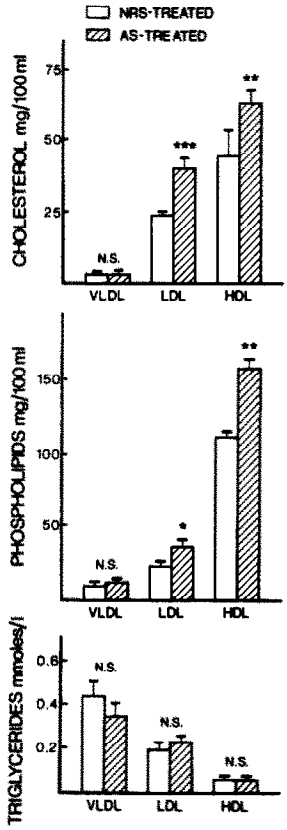

Fig.2. Cholesterol, phospholipid and triglyceride concentrations in plasma VLDL, LDL and HDL of rats given either anti-hepatic lipase serum (n = 10, hatched columns) or normal rabbit serum (n = 10, white columns) at 5 and 2.5 h before blood sampling. The bars indicate SEM. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ for the difference between group means. N.S. = not significant.

4. Discussion

The heparin-releasable hepatic lipase of rat has been recently shown to be located on the luminal surface of the endothelial cells lining the liver sinusoids [1]. Such a localization is consistent with the extremely rapid release of the enzyme by heparin. Furthermore, the close contact of the enzyme with circulating blood suggests that the lipase has a physiological role in the removal of plasma lipoproteins or of their constituent lipids, the enzyme being thus functionally analogous to lipoprotein lipase which is located in the vascular endothelium of extrahepatic tissues.

The present results support the above hypothesis. Inhibition of HEL by a specific antiserum in vivo was followed by significant increases of cholesterol and phospholipid concentrations in LDL and HDL. On the other hand, the HEL antiserum did not have any effect on triglyceride concentration in the lipoproteins and also the protein concentration in LDL (not measured in HDL) remained unaffected. These findings suggest that HEL could be an enzyme which by

some mechanism removes part of cholesterol and phospholipids from LDL and HDL particles during their passage through the liver. If apoprotein is not simultaneously removed the density of the lipoprotein particles must increase in this process. This might involve transformation of LDL_1 to LDL_2 and of HDL_2 to HDL_3. Opposite changes would thus be produced by HEL antiserum and it remains to be investigated whether this is the case. Anyway, by density criteria the lipoproteins of antiserum-treated rats were still LDL resp. HDL.

There is no direct proof for the claim that the observed rise of LDL and HDL cholesterol and phospholipids of the antiserum-treated rats was accounted for by the interference of the HEL-antiserum with the removal of these lipids but this seems to be the most plausible explanation. In a similar experiment anti-lipoprotein lipase serum given intravenously to roosters completely inhibited the catabolism of VLDL triglycerides and caused their accumulation into plasma [14]. That the hepatic endothelial cells have a role in the metabolism of LDL and HDL is also suggested by the finding that after administration of iodine-labeled LDL or HDL into rat the protein radioactivity accumulates into nonparenchymal liver cells rather than into hepatocytes [15].

Our results are also consistent with previous data indicating that cholesterol is taken up by the liver from both LDL and HDL [16,17]. Thus, the concentration of LDL (1.019–1.063 g/ml) esterified cholesterol was shown [16] in man to be lower in hepatic vein than in artery. A corresponding gradient could not be found for HDL cholesterol but this may be difficult to demonstrate because of the relatively small concentration of HDL cholesterol in the plasma and also due to splanchnic secretion of HDL. Hepatic cholesterol synthesis can be shown [17] in rats to be suppressed by infusion of either LDL or HDL, which must imply a hepatic uptake of cholesterol from these lipoproteins. There are no published data on the uptake of phospholipids from lipoproteins by the liver.

The mechanism by which the HEL could mediate the uptake of cholesterol from lipoproteins is not clear. One possibility is that the high phospholipase activity of HEL [18] could be responsible for first step in this process, viz. breakdown of the surface phospholipids of the lipoproteins. Hepatic nonparenchymal cells have also a high cholesterol ester hydrolase activity [19] but it is not known whether this is located in the endothelial cells. The finding that LDL apoprotein level was not influenced by HEL-antiserum is compatible with data indicating only minimal uptake and degradation of LDL protein by the liver [16,20]. A concept proposing the shuttle of LDL and HDL particles in the transport of cholesterol and phospholipids between peripheral tissues (including peripheral catabolism of triglyceride-rich lipoproteins) and the liver could also account for the discrepancies found earlier between the transport rates of cholesterol and apoproteins in LDL and HDL.

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