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ON THE METABOLIC FUNCTION OF HEPARIN-RELEASABLE LIVER LIPASE

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SUMMARY Intravenous administration of specific antibody against heparin-releasable liver lipase (liver lipase) induced a 75% inhibition of the enzyme activity in situ. Administration of the antibody resulted in an increase of high density lipoprotein (density range 1.050-1.13 g/ml; HDL_2) phospholipid levels (20% after 1 h; 54% after 4 h). Short-term (1 h) treatment with antibody had no significant effect on any of the other lipoprotein components. After long-term (4 h) treatment the free cholesterol level of HDL_2 and all components in the very low density lipoprotein (VLDL) + intermediate density lipoprotein (IDL) fraction were elevated (1.5-2.0 fold). In the low density lipoprotein (LDL) fraction only the phospholipid level was affected (increased by 72%). All lipid components in the HDL_3 fraction were decreased by the antibody treatment, but this decrease was only statistically significant for the cholesterolesters. The rate of removal of iodine-labeled high density lipoprotein (HDL) and LDL from serum was not affected by the antibody treatment.

These results suggest that liver lipase may promote phospholipid removal in vivo and show that a lowering of liver lipase in situ has profound consequences for serum lipoprotein metabolism.

The role of the heparin-releasable lipase from liver (liver lipase) in metabolism is unknown. The enzyme is secreted by isolated parenchymal liver cells[1,2] and is bound to non-parenchymal liver cells both in vitro[3] and in vivo[4]. This extracellular localization of the enzyme at the vascular endothelium suggests a role in serum lipoprotein metabolism. In order to obtain information on the metabolic function of liver lipase, we lowered the lipase activity in situ by injecting specific antibodies against the enzyme and studied the effects on serum lipoprotein levels and composition and on HDL and LDL catabolism.

MATERIALS AND METHODS

Preparation of antibodies against liver lipase. The antibody preparation was raised in rabbits against liver lipase purified from postheparin plasma or from heparin-containing liver perfusates. The antiserum inhibits liver lipase completely, and does not affect lipoprotein lipase from extrahepatic tissues[5] (see also Table I), nor serum lecithin cholesterol acyltransferase (LCAT) activity (unpublished

Abbreviations: VLDL, very low density lipoprotein (density <1.006 g/ml); IDL, intermediate density lipoprotein (density range 1.006-1.019 g/ml); LDL, low density lipoprotein (density range 1.019-1.050 g/ml); HDL_2, high density lipoprotein (density range 1.050-1.13 g/ml); HDL_3, high density lipoprotein (density range 1.13-1.21 g/ml); LCAT, lecithin cholesterol acyltransferase.

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observation). The γ-globulin fraction of the antiserum was isolated by $(NH_4)_2SO_4$ precipitation and protein A affinity chromatography[6]. The isolated γ-globulins were dissolved in 0.9% NaCl to a concentration of 27 mg protein/ml. 1 ml of this preparation (anti-liver lipase) inhibits 13.500 mU of liver triacylglycerol hydrolase, when preincubated with the enzyme for 5 min at 37°C. With the assay used 1 ml of postheparin rat plasma contains about 450 mU of hepatic triacylglycerol hydrolase, measured as described earlier[3].

Isolation and labeling of serum lipoproteins. Rat serum lipoproteins were separated by density gradient ultracentrifugation, as described before[7]. In some experiments (see Table III), in order to separate two subclasses of HDL, the serum was brought to a density of 1.30 g/ml with solid KBr and overlayered with salt solutions of 1.21, 1.050 and 1.006 g/ml. The proportions of the different solutions, the centrifugation and the method of tube slicing were as described in ref. 8. This method allows the isolation of 4 lipoprotein classes containing HDL_3, HDL_2, LDL and VLDL plus IDL. In other experiments (Table II) the serum was brought to a density of 1.21 g/ml and overlayered with salt solutions of 1.050, 1.019 and 1.006 g/ml. This gradient allows the isolation of HDL_2, LDL, IDL and VLDL[7]. LDL and HDL_2 apoprotein were labeled with ^{131}I or ^{125}I exactly as described before[7], using the ICl method. Triglycerides, cholesterol(esters), phospholipids and protein were determined with standard laboratory methods (c.f. ref. 7).

In all experiments male Wistar rats were used, after an overnight fast (body weight 200-220 g). Acetone-ether powders were prepared from rat tissues[9].

The results were statistically evaluated using the Student's t-test (unpaired, 2-tailed).

TABLE I

EFFECT OF TREATMENT WITH ANTI-LIVER LIPASE ON LIPOPROTEIN LIPASE AND LIVER LIPASE

Experiments in vitro. Aliquots of extracts of acetone-ether powders of rat hearts, and adipose tissue and purified liver lipase were incubated with γ-globulins of a non-immunized rabbit (control γ-globulins) or anti-liver lipase (1 mg of protein). After 1 h at 0°C lipoprotein lipase or liver lipase activities were measured as described in refs. 9 and 3, respectively. The results of 2 different experiments are given.
Experiments in vivo. Acetone-ether powders were made of the livers from 6 rats injected with either control γ-globulins or anti-liver lipase (c.f. Table III). In the acetone-powder homogenates liver lipase activities were measured. Activities are expressed as mU lipase activity/liver ± S.E.M.

	Enzyme activity (mU) after incubation with		% inhibition
	control-γ-globulins	anti-liver lipase	
In vitro			
Heart lipoprotein lipase	0.88	0.89	- 1
	0.42	0.43	- 2
Adipose tissue lipoprotein lipase	12.2	11.3	7
	3.5	3.4	2
Purified liver lipase	187	0.7	99.6
	192	1.2	99.4
In vivo			
Rat liver lipase(mU/liver)	3936±352 (n=3)	1017±70 (n=3)	75

RESULTS

A lowering of liver lipase *in situ* was achieved by the intravenous injection of anti-liver lipase into rats. The antibody preparation used gives a complete inhibition of liver lipase *in vitro* and does not affect lipoprotein lipase from rat heart or adipose tissue (Table I). Injection of the rats with anti-liver lipase results in a 75% reduction of lipase activity in the livers (Table I).

1 h after the administration of antibody a small (20%) but significant increase of the HDL_2-phospholipid level was found (Table II). None of the other components in the separated lipoprotein classes was significantly affected by the antibody treatment. In order to see whether the HDL-phospholipid content could be further increased by prolonged treatment with antibody, rats were injected twice with anti-liver lipase over a 4 h period. Again the HDL-phospholipid level was affected significantly ($P<0.001$), being now increased by 54% (Table III).

The concentration of free cholesterol in the HDL_2 fraction also increased significantly, while the protein and triglyceride levels were unchanged. In the LDL fraction only the phospholipids were elevated, while in the VLDL fraction all components were increased by a factor 1.5 to 2.0 in the antibody-treated rats, if compared to rats treated with control γ-globulins. In the HDL_3 fraction, however, all components tended to be higher in the control group, but only the decrease in cholesterolester concentration was statistically significant.

Since the protein contents of the LDL and HDL fractions were not affected by the antibody injection, we also studied whether the removal of LDL and HDL apoprotein from serum was affected by lowering of the liver lipase activity by

TABLE II

SERUM LIPOPROTEIN COMPOSITION 1 H AFTER THE INTRAVENOUS INJECTION OF ANTI-LIVER LIPASE (a) OR CONTROL γ-GLOBULINS (c)

6 rats were injected under nembutal narcosis with anti-liver lipase (27 mg of protein) or the same amount of control γ-globulins. After 1 h the rats were killed and the blood collected. Serum lipoproteins were separated and the concentrations of the lipoprotein components were determined. The results are given in µg's/ml serum ± S.E.M.

	VLDL		IDL		LDL		HDL_2	
	c	a	c	a	c	a	c	a
(µg/ml)								
Protein	76±22	54±10	41±7	30±8	134±10	130±10	507±32	611±58
Triglyceride	227±62	228±115	22±5	19±7	13±5	11±6	10±3	19±3
Phospholipids	60±14	49±18	35±6	30±5	144±10	175±8	689±26	842±45*
Total cholesterol	71±18	51±9	n.d.	n.d.	212±45	223±38	523±39	647±48

* $P<0.05$
n.d., not determined

TABLE III

SERUM LIPOPROTEIN COMPOSITION 4 H AFTER THE INTRAVENOUS INJECTION OF ANTI-LIVER LIPASE (a) OR CONTROL γ-GLOBULINS (c)

10 rats were injected intravenously under light ether anaesthesia with anti-liver lipase or control γ-globulins (27 mg of protein each). After 2 h the rats received the same dose again. 4 h after the first injection the rats were killed and serum lipoproteins were separated, as described under Materials and Methods. The results are given in μg of the different components/ml serum ± S.E.M. Cholesterolesters were calculated as the difference between total cholesterol and unesterified cholesterol.

	VLDL plus IDL		LDL		HDL_2		HDL_3	
	c	a	c	a	c	a	c	a
(μg/ml)								
Protein	72±13	112±7*	113±27	127±11	642±48	716±36	n.d.	n.d.
Triglyceride	287±62	531±82*	38±8	31±6	21±7	20±2	9±7	4±2
Phospholipids	115±30	221±15*	110±16	189±24*	579±42	894±28***	74±9	59±6
Cholesterol (free)	35±8	69±4**	39±5	52±7	88±8	126±7**	10±2	4±2
Cholesterolesters	45±7	64±6	102±12	108±14	314±59	382±16	39±7	18±2*

* P<0.05
** P<0.01
*** P<0.001
n.d., not determined

antibody treatment. Fig. 1 shows that the serum decay of iodine-labeled HDL and LDL was not influenced by the injection of antibody. The iodine-label in both fractions is almost exclusively present in the protein moiety of these lipoproteins, so that it can be concluded that the removal of HDL and LDL apoprotein from serum is not a liver lipase-dependent process.

DISCUSSION

This paper shows the effects of *in situ* lowering of liver lipase induced by injection of anti-liver lipase on the components of several lipoprotein classes. The primary effect is a 20% increase of the HDL-phospholipid concentration (present within 1 h after antibody injection). After 4 h a further elevation (54%) in HDL-phospholipids is observed. At this time the free cholesterol content of the HDL fraction is also enhanced.

Liver lipase exerts a number of acylglycerol hydrolase activities *in vitro* amongst which phospholipase A_1 activity[5,10,11]. Therefore the progressive rise in HDL-phospholipids may be due to impaired phospholipid removal due to inhibited liver lipase activity. The important role of the liver in HDL-phospholipid catabolism was recently demonstrated[12] to be in line with earlier studies on the turnover of total serum phospholipids[13,14]. Liver lipase may be the key-enzyme in this process. This idea was strengthened by the finding that

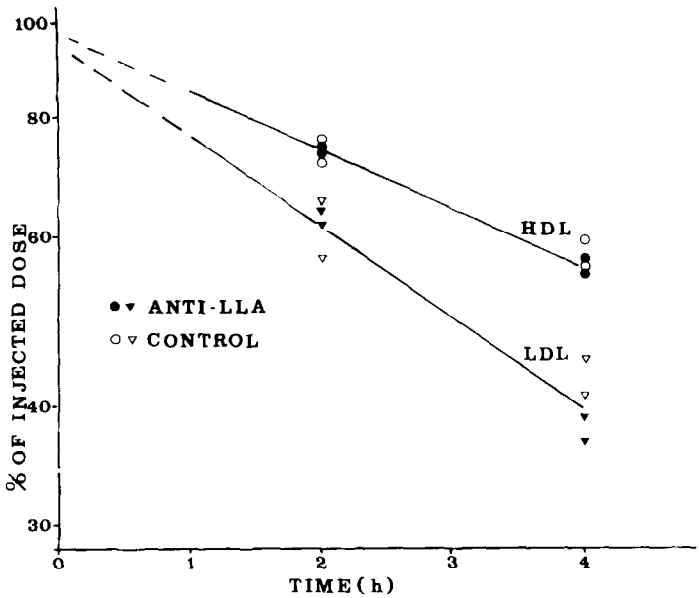

Fig. 1. Decay of serum radioactivity after intravenous injection of iodine-labeled LDL or HDL into rats treated with anti-liver lipase (closed symbols) or control γ-globulins (open symbols). The values are given as % of the injected dose. For details see Materials and Methods and the legend to Table III.

rats treated with anti-liver lipase and injected intravenously with ^{32}P-phospho lipid labeled HDL showed a higher serum/liver radioactivity ratio than rats treated with γ-globulins from control rabbits (unpublished observation). On the other hand the catabolism of the protein moieties of HDL and LDL seems to take place by a different, liver lipase-independent, mechanism (see Fig. 1).

Serum HDL can take up cholesterol from the cholesterol pool in a variety of tissues[15,16]. Therefore it is conceivable that secondary to the increase of HDL_2-phospholipids, cholesterol is taken up from tissues, leading to the observed increase in HDL_2 free cholesterol content. Glomset initially suggested that cholesterol is transported from extrahepatic tissues to the liver by way of serum HDL[16]. In this hypothesis LCAT plays a key-role with lecithin-rich HDL as preferred substrate. Recent work from our laboratory suggests an important function for the non-parenchymal liver cells in hepatic lipoprotein bound cholesterol(ester) uptake[17,18]. As liver lipase is located at the external surface of these non-parenchymal liver cells[3,4], this enzyme could be important for the hepatic uptake of lipoprotein-bound cholesterol as well as phospholipids (see also ref. 12).

Since HDL_2 protein and triglyceride levels are not affected by antibody treatment, the composition of the HDL_2 particles from the antibody treated

rats differs from the composition of the control animals. 4 h after treatment with antibody (but not after 1 h) all components in the VLDL plus IDL fraction are elevated. It is concluded (compare also the VLDL composition derived from the data given in Table II) that only the concentration of VLDL particles, of unchanged composition, is increased by treatment with anti-liver lipase. This increased level of VLDL can be explained in several ways. Liver lipase may be directly involved in the clearing of VLDL so that under conditions of low liver lipase activity the degradation of VLDL is impaired. Alternatively, VLDL catabolism may be modulated by impairment of HDL degradation. This second possibility seems to be more consistent with our results. A direct effect on VLDL removal would presumably be observed already during the first hour after antibody administration, as VLDL-triglyceride turnover is very rapid ($t_{\frac{1}{2}}$ of 2.4 min)[19]. This early effect was not found, however (see Table II). The data presented in this paper, together with recent studies from this and other laboratories, support the concept that VLDL, LDL and HDL metabolism are closely interrelated[20-22]. How an effect on HDL catabolism can interact with VLDL catabolism is not clear yet. A possible mechanism has been discussed elsewhere[23]. In contrast to the increased concentrations of several components of VLDL, LDL and HDL_2 all lipid components in the HDL_3 fraction seem to decrease in the antibody-treated rats (Table III). There is no clearcut explanation for this observation, but it suggests that liver lipase catalyzes the conversion of HDL_2 into HDL_3 (comp. also ref. 23).

After this study was completed, Kuusi et al.[24] published experimental data, using the same model system. Most of their results are consistent with our data. The dissimilarities with our work (e.g. they found no effect on the triglyceride-rich lipoproteins) may be due to differences in the feeding conditions of the rats.

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