A role for hepatic lipase in chylomicron and high density lipoprotein phospholipid metabolism

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Abstract The rate of removal of phosphatidylethanolamine and phosphatidylcholine from the plasma of rats treated with antiserum to hepatic lipase was measured. The hepatic lipase antiserum was injected intravenously into animals prior to injection of ^{32}P-labeled chylomicrons or ^{32}P-labeled high density lipoproteins. In experiments in which ^{32}P-labeled chylomicrons were injected, antiserum treatment inhibited removal of [^{32}P]phosphatidylethanolamine from chylomicrons, and the unlabeled serum phosphatidylethanolamine levels increased 2–2.5-fold in 30 min. In contrast, hepatic lipase antiserum had no significant effect on the clearance of chylomicron [^{32}P]phosphatidylcholine or on unlabeled phosphatidylcholine concentrations in serum after injection of chylomicrons. In experiments in which ^{32}P-labeled high density lipoproteins were injected, the inhibitory effect of the antiserum on the rapid removal of [^{32}P]phosphatidylethanolamine from the circulation was even more marked than its effect on the removal from chylomicrons. The removal of high density lipoprotein phosphatidylcholine on the other hand was unaffected by the antiserum, although a moderate increase in serum phosphatidylcholine concentration was seen. In antiserum-treated rats injected with ^{32}P-labeled chylomicrons or high density lipoproteins, hepatic [^{32}P]phosphatidylethanolamine radioactivity was decreased. Significantly more [^{32}P]phosphatidylethanolamine was recovered from blood plus liver in the antiserum-treated rats, indicating that the antiserum inhibited the overall degradation of injected [^{32}P]phosphatidylethanolamine. The data suggest that phosphatidylethanolamine is a preferred substrate for hepatic lipase in the metabolism of chylomicron and high density lipoprotein phospholipid.—**Landin, B., Å. Nilsson, J-S. Twu, and M. C. Schotz.** A role for hepatic lipase in chylomicron and high density lipoprotein phospholipid metabolism. *J. Lipid Res.* 1984. **25:** 559–563.

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Although phosphatidylcholine (PC) is the predominant phospholipid in chylomicrons, 10–15% of the phospholipid in these particles is phosphatidylethanolamine (PE) (1). In vitro, lipoprotein lipase can hydrolyze the 1-ester bond of both phospholipids (2, 3). During the intravascular metabolism of chylomicrons, a significant part of the polar components, including phospholipid, is transferred to high density lipoproteins (HDL) (4, 5). These HDL phospholipids are resistant to hydrolysis by lipoprotein lipase (6), but are actively hydrolyzed by hepatic lipase in vitro (7). Recently chylomicron PE was found to be rapidly cleared from plasma, although a significant part was transferred to denser lipoproteins before clearance (8). In view of the high affinity of hepatic lipase for PE in vitro (9), we have considered that the clearance of PE and PC in vivo may be due to the combined action of lipoprotein lipase on chylomicrons and of the hepatic lipase on HDL to which the polar chylomicron surface components have been transferred. To test this hypothesis we investigated the effects of antiserum against hepatic lipase on the metabolism of chylomicron and HDL phosphatidylethanolamine and phosphatidylcholine in vivo.

METHODS

^{32}P-Labeled chyle was obtained by feeding 2 mCi of sodium [^{32}P]phosphate (3–7 mCi/mg P) (Amersham) and 0.5 ml of corn oil to rats with thoracic duct drainage (10). Chylomicrons were isolated by adjusting the density of the lymph to 1.063 g/ml with KBr/NaCl (d 1.35 g/ml), layering under saline (d 1.006 g/ml), and then centrifuging at average 78,000 *g* for 120 min. The chylomicrons thus obtained were suspended in 0.15 M NaCl.

^{32}P-Labeled HDL was obtained by incubating 5 ml of ^{32}P-labeled chylomicrons, containing 46 mg of TG, 5.5 mg of PC, and 1.5 mg of PE (Expt. A) or 162 mg of TG, 10.1 mg of PC, and 0.88 mg of PE (Expt. B), with 10 ml of fresh rat serum for 2 hr at 37°C. Lipoproteins with

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; HDL, high density lipoproteins (d 1.063–1.21 g/ml); TG, triacylglycerols; FFA, free fatty acid.

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d < 1.063 g/ml were removed by centrifuging the mixture adjusted to this density at average 195,000 *g* for 36 hr before isolating the HDL by recentrifuging at d 1.21 g/ml at the same speed for another 36 hr. The top fraction obtained was dialyzed against 0.15 M NaCl before use. All centrifugations were performed in an MSE 6 × 14 swing-out rotor at 10°C.

The purification of hepatic lipase and preparation of antiserum to the pure protein have been described (11). Briefly, rat liver hepatic lipase purified about 60,000-fold to a specific activity of 30,000 μmol FFA/hr per mg of protein against a triolein substrate was used to raise antibody in a rabbit. This antiserum completely inhibited the hepatic lipase activity in the supernatant fraction of liver homogenates and also the enzyme activity released from liver by perfusion with heparin (11). Antibody titer was calculated from its inhibition of pure hepatic lipase activity with an emulsified triglyceride substrate; 60 μl of antiserum was able to inactivate all the hepatic lipase activity from 1 gram of liver tissue, approximately 1 unit of lipase activity. A unit of enzyme activity is defined as the release of 1 μmol FFA/min per mg of protein from an emulsified triglyceride substrate (11). Since only the fraction of hepatic lipase on the cell surfaces would be inactivated by circulating antibody, the antiserum dose, 200–250 μl, was calculated to be in considerable excess. Monospecificity of the antiserum was evidenced by the observation that, using a hepatic lipase antiserum immunoadsorbent column, a single band was obtained from liver extracts on SDS electrophoresis (11).

Male Sprague-Dawley rats, weighing 100–150 g, fasted overnight, were used. Rabbit antiserum to rat hepatic lipase or an equivalent volume of serum from a nonimmunized rabbit was injected intravenously 5 min before the injection of 0.3 ml of ^{32}P-labeled chylomicrons or 0.5 ml of ^{32}P-labeled HDL. Thirty min after the lipoprotein injection the animals were killed by aortic bleeding and serum was collected. Two series of experiments using different batches of ^{32}P-labeled chyle were performed. In experiment A, 250 μl of antiserum or control serum was injected as described above followed by chylomicrons containing 2.8 mg of TG, 0.33 mg of PC (1.18×10^6 cpm) and 92 μg of PE (2.44×10^5 cpm) or HDL containing 0.33 mg of PC (4.9×10^5 cpm) and 51 μg of PE (3.83×10^4 cpm). The animals were kept under light ether anesthesia and blood was collected from the tail into tared test tubes at specified intervals. At the end of the experiment, i.e., 30 min after the lipoprotein injection, the animals were killed as described above. In experiment B, 200 μl of antiserum or control serum was injected followed by chylomicrons containing 9.7 mg of TG, 0.61 mg of PC (5.38×10^5 cpm) and 53 μg of PE (2.00×10^5 cpm) or HDL containing 0.28 mg of PC (9.09×10^4 cpm) and 11 μg of PE (1.86×10^4 cpm). In this case the animals were allowed to wake up after the injection and were again anesthetized immediately before the aortic puncture.

Triacylglycerols were estimated by an enzymatic method (Boehringer-Mannheim GmBH) and choline-containing phospholipids were estimated by a phospholipase-choline oxidase method (Phospholipids B-test. Wako Chemicals, Osaka, Japan). PE was determined by fluorimetry after conversion to its fluorescamine derivative. The lipid extract from 50 μl of serum was reacted with fluorescamine according to Schmid, Pfeiffer, and Schmid (12). The reaction was terminated by adding 2 ml of chloroform, 1.5 ml of water, and 1.5 ml of methanol. The upper phase was discarded and the lower phase was washed twice with methanol–water–chloroform 48:47:3. Then the chloroform phase was taken to dryness and redissolved in spectroscopically pure ethanol. Fluorescence was determined in an Aminco Bowman spectrophotofluorimeter (excitation wavelength 395 nm, emission wavelength 468 nm). Similar results were obtained when the reaction mixture was subjected to thin-layer chromatography, and the fluorescence of the dominant spot seen under UV light, i.e., the PE fluorescamine derivative, was determined after extraction according to Schmid et al. (12).

Lipids in blood, serum, and liver samples were extracted with chloroform–methanol 1:1 and the extracts were washed as described earlier (10). The lipid extracts were dried under nitrogen, redissolved in chloroform, and subjected to thin-layer chromatography on silica gel G plates developed in chloroform–methanol–water–acetic acid 65:25:4:4. The PC and PE spots were scraped into counting vials; 1 ml of methanol–water 1:1 was added and the vessels were vortexed before adding 10 ml of toluene–Instagel (Packard Instruments Inc.) 1:1. Radioactivity was determined in a Packard TriCarb Liquid Scintillator. The data are expressed as % injected dose. The total blood weight, calculated as 8% of total weight, was 9.2–10.9 g (mean, 10 g). The total serum volume was calculated at 4% of body weight.

Values presented are the means ± SEM for the number of animals indicated. Statistical evaluation of the data was performed using Student's *t*-test.

RESULTS

Fig. 1 shows the marked difference in the rate of disappearance of chylomicron ^{32}P-PE and ^{32}P-PC from the plasma of the control rats. A large part of both ^{32}P-PC and ^{32}P-PE, 40% and 60%, respectively, was eliminated rapidly within the first 2 min; thereafter the clearance of ^{32}P-PC was notably slower than that of ^{32}P-PE.

The antiserum against hepatic lipase caused inhibition

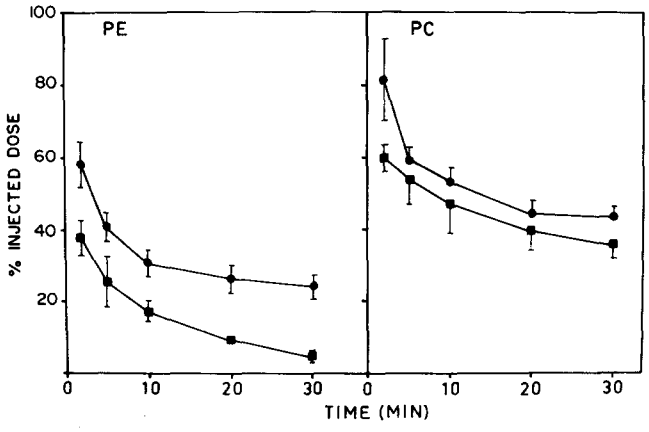

Fig. 1. Effect of hepatic lipase antiserum on clearance of chylomicron [^{32}P]phosphatidylethanolamine and [^{32}P]phosphatidylcholine. Rats were injected with antiserum against hepatic lipase (● —— ●) or control serum (■ —— ■) 5 min prior to the chylomicron administration as described for Expt. A in the Methods section. The animals were kept under light ether anesthesia and blood samples were taken from the tail at specified intervals. Values are the means ± SEM for three animals.

of chylomicron ^{32}P-PE clearance at all time intervals (Fig. 1). However, even in the presence of antiserum to hepatic lipase, a significant part of both ^{32}P-labeled chylomicron phospholipids was still cleared during the first 5 min. The clearance of ^{32}P-PE was thereafter significantly inhibited, and at 30 min 25–30% remained in the plasma of antiserum treated rats as compared to 2.5% in the control animals. The effect of the antiserum on chylomicron ^{32}P-PC clearance was much less marked than in case of ^{32}P-PE. Indeed, there was no significant inhibition of plasma ^{32}P-PC clearance at any time interval (Fig. 1, **Table 1**).

When ^{32}P-labeled HDL was injected, the initial removal of ^{32}P-PE was less rapid than that observed with labeled chylomicrons (**Fig. 2**). But still, in the control animals, the clearance of ^{32}P-PE exceeded 80% in 30 min. The inhibition of the ^{32}P-PE removal of hepatic lipase antiserum was very marked, with 50–60% remaining in blood after 30 min. In contrast, there was again no significant inhibition by the antiserum of the ^{32}P-PC removal (Fig. 2, Table 1).

The plasma PE concentrations were markedly increased 30 min after the injection of lipoproteins into animals pretreated with antiserum (**Table 2**). The rise in serum PE concentration exceeded the amounts that could possibly have been contributed by the injected lipoproteins. This is very clear since the total PE contained in the injected lipoproteins ranged from 10–90 μg and the mean increases in the total serum PE amounted to almost 200 μg. A significant increase in serum PC was seen only after HDL injection, while a considerable degree of variation between individuals obscured any possible difference between the antiserum-treated and control group after chylomicron injection. However, the increase in serum PC caused by the antiserum also exceeded the amount contributed by the injected lipoproteins. There were no differences in plasma triacylglycerol levels between the different groups (Table 2), indicating that the antiserum had no significant effect on the clearance of either chylomicron or HDL triacylglycerols.

In rats injected with either chylomicrons or HDL, the hepatic ^{32}P-PE radioactivity in rats injected with antiserum was lower than in the control rats (Table 1) although the differences were not as pronounced as the marked differences in removal of ^{32}P-PE from plasma. The recovery of ^{32}P-PE from blood plus liver was higher in the antiserum treated groups (Table 1), indicating that the proportion of overall ^{32}P-PE degradation was less in these groups. The average recovery of ^{32}P-PE after injection of chylomicrons plus antiserum was lower than after in-

TABLE 1. Effect of hepatic lipase antiserum on tissue distribution of ^{32}P-labeled phosphatidylcholine and phosphatidylethanolamine

		% of Injected Radioactivity			
		Chylomicrons		HDL	
Organ	Antiserum	PC	PE	PC	PE
Serum/blood	+	57.0 ± 3.1	27.5 ± 1.8[a]	58.3 ± 2.5	52.2 ± 3.3[a]
	−	52.3 ± 4.6	11.3 ± 2.0	53.6 ± 2.5	18.3 ± 1.0
Liver	+	12.6 ± 0.4	27.7 ± 2.3[b]	12.0 ± 0.7	27.1 ± 2.1[b]
	−	14.3 ± 0.8	37.2 ± 2.1	10.9 ± 0.3	43.9 ± 1.9
Serum/blood + liver	+	69.7 ± 2.9	55.3 ± 1.8[c]	70.4 ± 2.6	79.3 ± 4.0[b]
	−	66.6 ± 4.2	48.5 ± 1.8	64.5 ± 2.4	62.2 ± 1.7

Tissues were obtained from animals treated as described in the Methods section 30 min after lipoprotein injection. Values are means ± SEM for the nine animals of experiments A and B.
[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.

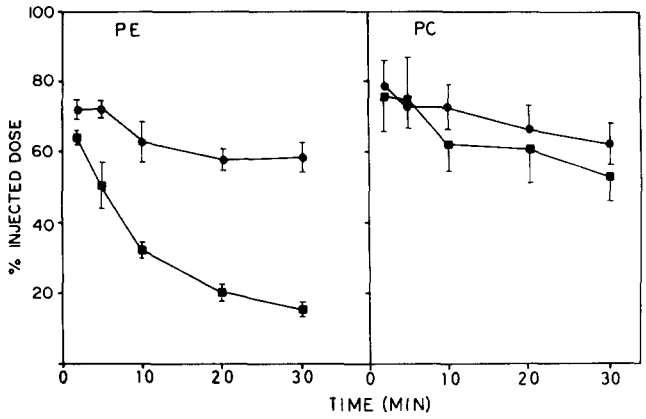

Fig. 2. Effect of hepatic lipase antiserum on clearance of HDL [^{32}P]phosphatidylethanolamine and [^{32}P]phosphatidylcholine. Animals were treated as described in the Methods section (Expt. A) and in the legend to Fig. 1. Values are the means ± SEM for three animals.

jection of HDL plus antiserum, indicating that a higher proportion of ^{32}P-PE in chylomicrons than in HDL was catabolized in some way unaffected by hepatic lipase antiserum, probably by lipoprotein lipase. The differences in total recovery of ^{32}P-PC between antiserum-treated and control animals were not significant (Table 1).

DISCUSSION

The present study indicates that hepatic lipase has an important role in the metabolism of chylomicron and HDL PE. Hepatic lipase antiserum significantly decreased both the amount of ^{32}P-PE eliminated from serum and that recovered in the liver 30 min after lipoprotein injection (Table 1). During the same time period no significant effect of the antiserum on the metabolism of chylomicron or HDL ^{32}P-PC could be detected. The normal elimination rate of chylomicron PC is, however, much slower than that of PE and moderate changes in the clearance of PC would thus be more difficult to detect. Further experiments over longer time periods will be needed for a quantitative estimation of the amount of chylomicron PC that is metabolized by hepatic lipase.

Administration of hepatic lipase antiserum not only inhibited the clearance of chylomicron or HDL ^{32}P-PE, but also caused the serum level of unlabeled PE to increase more than 100% which is far more than could be accounted for by the injected PE. A significant increase in unlabeled PC concentration was detected only in animals injected with antiserum and HDL, and this increase amounted to only about 15%. This indicates further that serum PE turns over more rapidly, perhaps up to 10 times faster, than serum PC. The role of PE in the transport of phospholipid fatty acids may thus be more important than is apparent from the low plasma PE concentration.

In experiments where chylomicrons were injected, there was a rapid initial clearance of a part of both PE and PC which cannot be inhibited by hepatic lipase antiserum (Fig. 1). In our earlier experiments (8) there was also a rapid initial disappearance of both PE and PC from the injected chylomicrons, which exceeded the appearance of these phospholipids in denser lipoproteins. It is likely that the effect of lipoprotein lipase on chylomicrons contributes to this initial disappearance. The proportion of chylomicron phospholipids that is metabolized by the lipoprotein lipase pathway is difficult to determine quantitatively, but a rough estimation from the initial slope of the curves for PE and PC clearance from the antiserum-treated animals (Fig. 1) indicates that it could amount to about 40% of the injected PE and 20–30% of the PC. It should be noted that hepatic lipase antiserum inhibits chylomicron PE clearance at a time when the substrate for the enzyme could be phospholipids in either chylomicrons, released chylomicron surface material which has not been integrated into HDL particles, or HDL particles to which chylomicron phospholipids have been transferred. Although the data indicate an important role for hepatic lipase in the metabolism of chylomicron phospholipids, they do not provide information as to which

TABLE 2. Effect of hepatic lipase antiserum on serum lipid levels of animals injected with lipoprotein fractions

Lipoprotein	Antiserum	TG	PC	PE
		mg/ml	*mg/ml*	*μg/ml*
Chylomicrons	+	1.11 ± 0.15	1.68 ± 0.07	65.8 ± 6.7[a]
	−	0.99 ± 0.15	1.59 ± 0.09	28.5 ± 2.7
HDL	+	0.70 ± 0.06	1.53 ± 0.04[b]	68.4 ± 4.8[a]
	−	0.59 ± 0.04	1.34 ± 0.04	28.1 ± 2.1

Serum was obtained 30 min after lipoprotein injection. The experiments are described in the Methods section. Values are means ± SEM for the nine animals of experiments A and B.
[a] $P < 0.001$.
[b] $P < 0.01$.

particles are the physiological substrate for the enzyme. Our earlier data (8) indicate, however, that the transfer of remaining phospholipids to denser lipoproteins during the metabolism of chylomicrons is very rapid, and it is extremely likely that the data obtained 5–30 min after injection represent the metabolism of phospholipids in dense lipoproteins, presumably HDL. The findings in the present study are thus compatible with the idea that phospholipid-rich HDL is the preferred substrate for hepatic lipase (7, 13–16).

A decrease in hepatic ^{32}P-PE radioactivity was seen in the antiserum-treated rats, but it was less than expected from the marked effects of antiserum on removal of ^{32}P-PE from the plasma. However, the total recovery of PE radioactivity in serum plus liver was larger in the rats that had been treated with antiserum than in the controls. It is thus possible that most of the hepatic ^{32}P-PE in the controls had been formed in a deacylation-reacylation cycle of chylomicron PE, during which a significant part of the ^{32}P-2-lyso-PE formed had been degraded further rather than reacylated. In contrast, in the experiments with antiserum, more ^{32}P-PE was available in plasma for exchange reactions with liver phospholipids.

The finding that PE hydrolysis is greatly inhibited by the hepatic lipase antiserum whereas PC is virtually unaffected suggests that PE is a specific substrate for the hepatic lipase. This is in line with earlier findings by Ehnholm et al. (9) who noted that the hepatic lipase activity in human postheparin plasma was approximately 25-fold greater with PE as substrate than with PC.

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