

Influence of heparin on the removal of serum lipoprotein lipase by the perfused liver of the rat

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ABSTRACT Isolated rat livers were perfused with whole rat blood containing postheparin lipoprotein lipase (LPL) activity. LPL activity disappeared rapidly from the perfusate; the extraction ratio (portal vein–hepatic vein difference) was 0.70 for all time periods studied. Control experiments established that the disappearance of LPL was not due to non-specific inactivation in the apparatus or to the release of an inhibitor by the liver. The addition of heparin to the perfusate in suitable concentration (4 units/ml) almost completely blocked the disappearance of LPL activity from the perfusate. In addition to the perfusion experiments, we studied the effect of heparin on LPL activity when added to the LPL assay system. When heparin was added to the assay system containing fresh postheparin serum from rats, it stimulated LPL activity by about 70%. When heparin was added to postheparin serum which had been perfused through the liver, it stimulated LPL activity over 200%, but it did not restore LPL to its preperfusion value. These observations are compatible with a two-step inactivation system for LPL by the liver. The first step may involve a dissociation of a heparin–apoenzyme complex followed by destruction of the heparin. The second step may involve the removal of the apoenzyme of LPL.

SUPPLEMENTARY KEY WORDS lipoprotein lipase · heparin-apoenzyme complex · LPL inactivation · postheparin · inhibition · stimulation

AFTER THE INTRAVENOUS injection of heparin into animals and man, lipoprotein lipase (LPL) is released into the circulation and then disappears exponentially (1, 2). The site of release is considered to be the endothelial lining of tissue capillary beds which contain the enzyme. Robinson and Harris were the first investiga-

tors to present evidence that the peripheral capillary beds are an important source of LPL released by heparin injection (3). The liver vasculature may also be a source of postheparin lipase in the dog (4), the rabbit (5), and man (6).

The first experimental evidence that the liver is a site of removal or inactivation of LPL was presented by Jeffries (7) and later by Spitzer and Spitzer (8). In addition, the studies of Yoshitoshi et al. (1) showed that the rate of disappearance of postheparin LPL activity from the circulation is markedly slowed after hepatectomy. Connor and Eckstein evaluated the role of the liver in the removal of postheparin LPL activity in patients with Laënnec's cirrhosis and in dogs with hepatic necrosis (9). The exact mechanism by which the liver removes LPL activity from the blood is not known. Yoshitoshi et al. (1) demonstrated that liver homogenates are inhibitory to postheparin LPL activity. Mayes and Felts (10) found that acetone powder preparations of rat liver inhibited LPL activity from heart muscle. Wayne, Felts, and Harris have recently studied the removal of postheparin LPL activity by the liver in unanesthetized dogs. Large heparin doses administered intravenously were found to

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Abbreviations: LPL, lipoprotein lipase; FFA, free fatty acids.

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effectively block the exponential decline of LPL activity in the circulating plasma (11).

In this report we describe studies in a perfused rat liver system which were designed to elucidate further the role of the liver in the removal of LPL activity from the circulation. We also studied the effect of increased heparin concentrations on the removal process.¹ In addition, we have investigated the effect of heparin on the stimulation of LPL activity when added to postheparin serum obtained directly from rats and when added to postheparin serum from liver perfusates.

METHODS

Perfusion Experiments

Livers were obtained from male Long-Evans rats (340–360 g) which had been maintained on rat laboratory chow. Perfusions were carried out as previously described (12). The perfusate consisted of whole defibrinated rat blood that had been dialyzed overnight against Krebs–Henseleit buffer containing glucose, 3 mg/ml, and a mixture of amino acids, 0.5 mg/ml. After the livers were perfused for 10–15 min to allow the temperature and flow rate to stabilize, fresh, defibrinated postheparin rat blood was added to the perfusate in a ratio of approximately 1:1. The postheparin blood was obtained by direct heart puncture from ether-anesthetized rats that had been injected with heparin, 50 units/kg (Evans Medical Supplies Ltd., Liverpool, England) via the jugular vein 10 min earlier. After the addition of the postheparin blood to the perfusate, 5 min were allowed for mixing. In most experiments, the livers were then perfused for an additional 60 min. Blood samples for analysis were obtained either from the inflow cannula (portal vein) or from the inflow cannula and outflow cannula (hepatic vein) simultaneously.

In experiments in which heparin was added to the perfusion system, a solution of heparin in 0.15 M NaCl was mixed with the postheparin blood prior to its addition to the perfusate. The final concentration of heparin in the perfusion mixture was 4 units/ml.

Control experiments consisted of mixing serum from defibrinated rat blood which had been dialyzed overnight with equal parts of fresh postheparin serum. This mixture was circulated in the perfusion apparatus without a liver in the circuit. 5 min were allowed for mixing; thereafter, samples of the serum were periodically removed for analysis over a period of 60 min. Serum, rather than defibrinated blood, was used in these studies

to avoid lactic acid production from glucose which may have caused a pH change over the course of the study.

LPL Assay System

Samples of the perfusate were collected in ice-cold centrifuge tubes, centrifuged at 1,000 *g* for 15 min at 5°C, and the serum was removed. Blood samples from rats were collected in centrifuge tubes and defibrinated with wooden applicator sticks. The samples were then centrifuged at 1,000 *g* for 15 min at 5°C, and the serum was removed. Serum samples were assayed for LPL activity by a modification of Robinson's method (10). The assay system consisted of 0.75 ml of 1.35 M Tris buffer (pH 8.4), 2.25 ml of 15% (w/v) solution of bovine albumin (pH 8.4), 1.50 ml of triglyceride substrate consisting of 1 part 5% Intralipid (A. B. Vitrum, Stockholm, Sweden) which had been incubated at 37°C with 4 parts of fresh rat serum for 30 min, 1.0 ml of 0.025 M NH₄OH adjusted to pH 8.6 with HCl, 1.0 ml of test serum, and 0.5 ml of 0.15 M NaCl or heparin dissolved in 0.15 M NaCl. Incubations of the assay system were carried out at 37°C for 1 hr. Aliquots of the incubation mixture were removed at 0, 30, and 60 min for analysis. Lipase activity was assayed by titrating, in duplicate, the fatty acids liberated during the incubation according to the method of Dole as modified by Trout, Estes, and Friedberg (13). The assay appeared to be linear during the period of incubation. 1 unit of LPL activity is equivalent to 1 μ eq of FFA released per hr by 1 ml of serum.

RESULTS

Experiment 1a

Removal of LPL Activity by the Perfused Liver and Effect of Heparin Addition to the Perfusate (Fig. 1). When defibrinated postheparin blood was added to the perfused liver system, the LPL activity was rapidly removed from the circulating perfusate with an initial $t_{1/2}$ of approximately 15 min (L). This value is dependent on at least two factors: the efficiency of the liver mechanism for removal of enzyme activity and the fraction of the perfusate which traverses the liver per unit time. In the control studies (C), only serum containing LPL activity was circulated in the apparatus. There was little decline in enzyme activity over a 60 min period. The differences between the LPL values found in the control (C) and the liver perfusion studies (L) were significant at all time intervals.

In two studies, heparin was added to the defibrinated postheparin blood just before it was added to the perfused liver system to give a final concentration of 4 units/ml (L + H). Under these conditions the removal of LPL activity by the liver was almost completely blocked; the $t_{1/2}$ was 115 min.

¹ Throughout this paper the words disappearance, removal, and extraction have been used interchangeably when referring to a reduction of LPL activity in the perfusate.

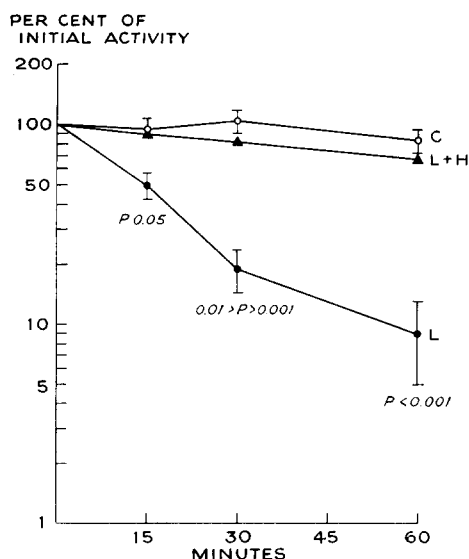


FIG. 1. Removal of LPL activity by the perfused liver and the effect of added heparin. *L*, perfused liver. In the five studies initial LPL activities were 4.55, 3.30, 2.33, 0.98, and 1.52 units. *L+H*, perfused liver with heparin addition to perfusate. In the two studies initial LPL activities were 6.62 and 9.98 units. *C*, control with no liver in the perfusion apparatus. In the four studies initial LPL activities were 2.10, 2.93, 1.21, and 3.02 units. *P* values are for comparison between *L* and *C*. Points represent mean values (\pm SEM where applicable). Tests of significant differences between mean percentages were carried out using Student's *t* test.

Experiment 1b

Extraction Ratio of LPL Activity by the Perfused Liver and Effect of Heparin Addition to the Perfusate (Table 1). The extraction ratio of LPL activity was determined by sampling the portal vein perfusate and the hepatic vein perfusate simultaneously. LPL activity determined in this way showed that the portal vein-hepatic vein extraction ratio was 0.70. After 30 min of perfusion, the LPL activity was so low in the perfusate from the hepatic vein that the values for an extraction ratio were unreliable; therefore, only the samples taken at the beginning of the perfusion ("0"-time) and at the 15-min points are presented.

The addition of heparin to the perfusate in a final concentration of 4 units/ml blocked the initial extraction of the LPL activity. The extraction ratio was found to have a negative value at 0 time undoubtedly due to the release of a small amount of lipase from the liver as a result of the heparin addition (14). Thereafter, the extraction ratio gradually increased with time and was 0.10 at 60 min.

Experiment 2

Investigation of the Possible Release of an Inhibitor of LPL Activity from the Perfused Liver. A liver was perfused for 1 hr with defibrinated blood that did not contain postheparin LPL activity. Aliquots of the serum from the

TABLE 1 EFFECT OF HEPARIN ON THE EXTRACTION OF LPL ACTIVITY BY THE PERFUSED RAT LIVER

Time of Perfusion	Experiment Number	LPL Activity in Serum from Portal Vein Cannula*	Additions to Perfusate	Extraction Ratio†
min				
0	1	4.55	None	0.70 \pm 0.014‡
	2	3.30		
	3	0.98		
	4	1.52		
15	2	1.66		0.72 \pm 0.087
	3	0.79		
	4	0.96		
0	5	6.62	Heparin (4 U/ml)	-0.09
	6	9.98		
15	5	5.98		0.02
	6	9.51		
30	5	5.30		0.04
	6	8.54		
60	5	4.18		0.10
	6	7.13		

* μ eq FFA released per ml serum per hr.

† Portal vein activity - Hepatic vein activity

Portal vein activity

‡ Mean \pm SEM.

perfusate were then added to samples of postheparin serum obtained directly from rats. LPL activity was then determined in mixtures of serum from the perfusate plus postheparin serum and in postheparin serum alone. There was no inhibition of LPL activity observed upon the addition of serum from the perfusate to the postheparin serum samples.

Experiment 3a

Stimulation of Serum LPL Activity by Heparin Addition to the Assay System (Table 2). A liver was perfused with defibrinated rat blood for 15 min, and fresh postheparin defibrinated blood was then added to the perfusate. Perfusate samples were removed 5 min later for analysis.

TABLE 2 STIMULATION OF SERUM LPL ACTIVITY BY HEPARIN ADDITION TO ASSAY SYSTEM

Source of LPL	Without Added Heparin	With Added Heparin (4 U/ml)
	μ eq FFA/ml serum/hr	
Postheparin serum before addition to perfusion*	3.9	7.6
Portal vein serum from perfusate†	1.7	5.1
Hepatic vein serum from perfusate†	0.39	1.9

* Corrected for potential dilution of LPL activity after addition to perfusion system.

† Simultaneous samples taken 5 min after addition of postheparin blood to the liver perfusate.

TABLE 3 PER CENT STIMULATION OF SERUM LPL ACTIVITY BY THE ADDITION OF HEPARIN TO THE ASSAY SYSTEM

Source of LPL	Heparin Addition to Assay	% Stimulation due to Heparin	P Value*
Postheparin serum from rats (3)†	4 U/ml	73 ± 18.7‡	—
Portal vein serum from perfusate (4)†	4 U/ml	247 ± 35.0‡	P < 0.02
Hepatic vein serum from perfusate†	4 U/ml	278 ± 66.6‡	P = 0.05

* Calculated by comparison with values for fresh postheparin serum.

† Number of samples. In these studies values from samples taken at 0 time and at 15 min were used.

‡ Mean ± SEM.

Serum from the postheparin blood obtained directly from rats was assayed for LPL activity with and without the addition of heparin (4 units/ml) to the assay system. LPL activity increased from 3.9 to 7.6 μ eq FFA. Serum samples from the perfusate were also markedly stimulated by addition of heparin to the assay system, but heparin did not increase the LPL values to the level seen in the heparin-stimulated values for fresh serum.

Experiment 3b

Per Cent Stimulation of Serum LPL Activity by the Addition of Heparin to the Assay System (Table 3). Livers were perfused as described for experiment 3a. LPL activity was assayed in serum samples with and without the addition of heparin to the assay system (4 units/ml). The LPL activity of postheparin serum freshly obtained from rats 10 min after the intravenous injection of heparin was increased 73% by the in vitro heparin addition. In serum samples obtained from the perfused liver studies the stimulation was much greater. Serum samples obtained from the portal inflow were stimulated 237% by the heparin addition. The stimulation was 278% in serum samples obtained from the hepatic venous blood. The difference in the effect of added heparin on the stimulation of LPL activity in fresh postheparin plasma and on the LPL activity in serum samples obtained from the perfusate was statistically significant.

DISCUSSION

The exact form of the postheparin enzyme complex (LPL) in serum which hydrolyzes a triglyceride substrate to FFA and glycerol is not known with certainty. Korn's original studies on the tissue enzyme established that several factors may influence the rate of hydrolysis (15, 16). The nature of the triglyceride substrate is of great importance since triglyceride emulsions must be "activated" by a serum component before the reaction takes place. The most active serum component in increasing

the rate of reaction was α_1 -lipoprotein. Korn clearly showed that low concentrations of heparin can increase the activity of LPL obtained from ammonia extracts of acetone powders from rat heart muscle. He further demonstrated that activity was markedly reduced by protamine or by reaction of the enzyme with a bacterial heparinase. More recently Gartner and Vahouny have demonstrated heparin activation of a soluble form of LPL from homogenates of heart muscle (17). These studies suggest that heparin functions as a cofactor or prosthetic group for the enzyme. On the other hand, Brown, Boyle, and Anfinsen observed that addition of heparin (4×10^{-6} M) to human postheparin serum inhibited the enzyme activity (18).

One major source of postheparin LPL present in serum was established by the studies of Robinson and Harris who presented evidence that heparin displaces LPL from the endothelial surface of peripheral capillary beds (3). Crass and Meng have shown that optimum release by heparin of enzyme activity from the capillary bed of the heart is dependent on the presence of serum (19). Thus, the circulating enzyme may consist of a complex of apoenzyme, heparin, and a serum component which acts enzymatically on a triglyceride substrate activated with the same or a different serum fraction, possibly α_1 -lipoprotein.

Jeffries (7) demonstrated that postheparin blood lost "clearing activity" when perfused through a rat liver. His experiments were the first to show that the liver might play a role in the removal of LPL from the circulation after heparin injection. Other studies have also demonstrated that the liver may be important in the removal of LPL from blood (1, 9). The studies of Mayes and Felts showed that rat liver acetone powder preparations are able to inhibit completely the LPL activity from heart muscle acetone powder preparations (10). The action of the LPL inhibitor system was blocked by the addition of heparin in suitable concentrations. These observations led to the concept (10) that one phase of the inhibitor process might be the removal of heparin by a liver heparinase (20). On the other hand, work from several laboratories has shown that the liver can release LPL into the circulation after the intravenous injection of heparin (4-6). Our earlier studies showed that liver acetone powder preparations can markedly inhibit added LPL activity (10). However, the liver acetone powders also contain an inactive form of the enzyme which can be detected only in the presence of high concentrations of heparin.

In order to understand better the activation of LPL by heparin and the inactivation of LPL by the liver we studied the enzyme using an isolated perfused rat liver system. The results of experiments 1a and 1b showed that the perfused liver is very efficient in the inactivation of

LPL. The portal vein-hepatic vein extraction ratio of LPL activity was approximately 0.70. In the presence of relatively high concentrations of heparin, the extraction ratio was greatly reduced over the period of study. The initial LPL activities in these experiments were higher than the initial values for the perfusion studies without added heparin. This could have been the result of at least two factors: the in vitro stimulation of LPL activity by the added heparin and additional LPL released from the liver by the added heparin. The average initial extraction ratio of -0.09 suggests that some LPL activity was released by the liver when heparin was present in the perfusate at a concentration of 4 units/ml. This value would indicate that a minimum of 10% of the initial activity present in these studies came from the liver itself. In earlier studies of the release of LPL by heparin in the perfused liver, it was found that 4 units of heparin per ml of perfusate caused release of about 1.0 LPL unit (14). If this were the case in these studies, then this could represent from 10 to 15% of the activity present at 0 time. At later time intervals the contribution of the liver LPL to the values assayed is uncertain. The extraction ratio for later time intervals indicate a gradually increasing rate of removal, and after 60 min of perfusion the extraction ratio was 0.10. In other experiments (Fig. 1) we also showed that LPL in the perfusate is inactivated only slowly by the perfusion apparatus itself, since post-heparin serum which was circulated in the perfusion apparatus without a liver in the circuit showed only a 15% reduction in LPL activity after 60 min. Experiment 2 showed that the liver does not secrete a substance inhibitory to LPL activity.

The results reported here with an in vitro system are similar in many respects to a recent in vivo study in the dog (11). In that study the extraction ratios across the livers of two dogs were 0.68 and 0.42 as compared with a ratio of 0.70 observed here. The injection of relative large doses of heparin led to a marked reduction of the extraction ratio. The results from the dog experiments together with the results of the experiments presented here led us to propose a mechanism for the removal of LPL activity. The mechanism by which LPL activity is removed from the circulation by the liver may involve several steps. The rapidity of the interaction between the circulating LPL and the inactivation system implies that the LPL complex interacts with sinusoidal membranes which may contain the inactivating system. This inactivation could be a function of either the parenchymal cell surface or the endothelial cells of liver. This concept is consistent with an earlier observation that the specific activity of the inactivation system is highest in acetone powders prepared from membrane fractions of liver (21). If we assume that the active enzyme circulates as the heparin-apoenzyme complex, the first of the inactivation

reactions taking place at the membrane surface may be the dissociation of the heparin-apoenzyme complex by removal or binding of heparin followed by the destruction of heparin by a liver heparinase. We have shown that heparin in suitable concentrations blocks the inactivation system. High heparin concentrations could possibly block the dissociation of the heparin-apoenzyme complex by saturating a heparinase system. The second step of the process could involve the destruction or binding of the apoenzyme itself or the apoenzyme-serum component complex. This postulate is supported by the results of experiment 3b (Table 3). When heparin was added to postheparin serum obtained directly from rats, we observed a 73% increase of LPL activity. When heparin was added to serum which had been circulated in the perfusion apparatus, there was over a 200% stimulation of LPL activity. This would imply that the greater contact of the LPL heparin complex with the liver leaves a greater proportion of apoenzyme which can then be reactivated by the addition of heparin. Even though the percentage stimulation is markedly increased by heparin added to the perfusate, we did not restore the absolute LPL activity to the level originally present in the perfusate as indicated by experiment 3a (Table 2). From these findings it appears that there may be a process in liver which effects the removal of the apoenzyme.

The studies presented here suggest that postheparin LPL activity in serum from rats may exist as a complex which is not fully active, since the activity can be further increased by the in vitro addition of heparin. Upon perfusion through the liver, LPL activity decreases rapidly. LPL activity in serum from the perfusate can be markedly stimulated by the addition of heparin to the assay system although this activity cannot be restored to its initial value. The removal of LPL activity by the liver can be effectively blocked by increasing the concentration of heparin in the perfusate. These observations are compatible with a two-step removal mechanism in the liver: first, the removal of heparin and, second, the removal of apoenzyme.

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