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KINETIC STUDIES ON METABOLISM OF LIPOPROTEIN LIPASE *

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Since Hahn demonstrated the clearing of alimentary lipemia by the intravenous injection of heparin, many excellent studies have been done on lipoprotein lipase (LPL). In these studies, however, LPL activities were compared in normal and pathological conditions at a certain time after heparin injection. If the level of LPL activity in the circulating blood changes continuously after heparin injection, under the influences of production and inactivation of LPL, it may be much more useful, under certain circumstances, to measure the rate of change as well as the amount of such activity.

One of the authors reported previously (1) the method of estimating the release or production rate and inactivation rate of LPL after heparin injection. This report will present the data on the metabolism of LPL activity in blood after the intravenous injection of heparin in normal subjects and patients with liver diseases, obesity, and coronary sclerosis, and in some animals.

EXPERIMENTAL METHODS

Preparation of materials. Human subjects and experimental animals were fasted overnight before the experiment. Experimental animals (dogs and rabbits) were anesthetized with pentobarbital sodium (Nembutal) just before use.

Heparin injection. The doses of heparin injected were 0.1 mg per kg of body weight throughout the experiment. The injection was done rapidly into the antecubital vein in men and dogs and into the ear vein in rabbits.

Withdrawals of blood. At certain intervals after the injection, blood was withdrawn from the antecubital vein in human subjects, from the femoral vein in dogs, and by heart puncture in rabbits. The blood specimen was immediately added to 1/9 vol of 1/10 M sodium oxalate solution kept in an ice-water bath.

Assay of LPL activity. The test plasma was obtained by centrifuging the blood for 5 minutes at 4,000 rpm in the cold. The substrate consisted of 0.1 vol of 2.5% sesame oil emulsion (Fatgen),[1] 1 vol of human normal fasting plasma, 1 vol of 1/15 M phosphate buffer (pH 8.0), and 1 vol of physiological saline solution. The substrate was preincubated at 37° C for 30 minutes just before use.

Immediately after the mixing of 1 ml of test plasma with 1.5 ml of substrate, 1.0 ml of the mixture was pipetted into Dole's extraction mixture, and the remainder was incubated at 39° C for 30 minutes. The LPL activity was expressed by the difference of FFA concentrations (microequivalents per liter) in the mixture before and after the incubation. FFA was measured by Dole's method (2). The details of our method were discussed in the previous paper (1).

RESULTS AND DISCUSSION

LPL activity curve in normal human beings, dogs, and rabbits. Eight healthy men aged 25 to 35 years, 4 dogs, and 3 rabbits were used. Figures 1 and 2 show the changes of LPL activity as a function of time in human subjects and dogs, respectively. Similar changes of LPL activity were observed also in rabbits. The rapid rise in LPL activity was observed immediately after heparin injection, and the peak was after about 9 minutes in human subjects, about 8 minutes in dogs, and about 3 minutes in rabbits. Thereafter, the activity fell at a constant rate, linearly on the logarithmic scale.

If: *1*) heparin (H_0 milligrams per kilogram of body weight) is injected instantly; *2*) the disappearance of heparin from the circulating blood is governed by the rate constant a; *3*) LPL is produced in proportion to the amount of heparin in the blood (proportional constant c); and *4*) LPL is inactivated at the constant rate b, then the changes of LPL activity, L, in the circulating blood may be expressed as follows: $L = [cH_0/(a - b)](e^{-bt} - e^{-at})$. The validity of this equation was discussed in detail in our previous paper (1), and also may be judged from the fitness of the theoretical curve to experimental data in Fig-

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[1] Kindly supplied by Dainihon Seiyaku Co., Ltd., Ōsaka, Japan.

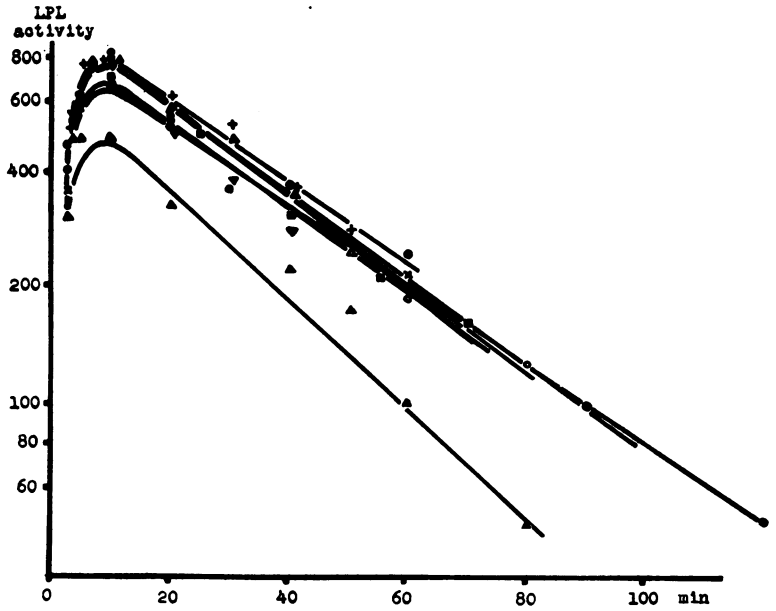

FIG. 1. THE CHANGES IN LIPOPROTEIN LIPASE (LPL) ACTIVITY AS A FUNCTION OF TIME AFTER THE INJECTION OF HEPARIN, 0.1 MG PER KG OF BODY WEIGHT, IN NORMAL SUBJECTS.

ures 1 and 2. The gradient of the declining curve may express the rate of inactivation of LPL, *b*.

The coefficient and exponential constants of the LPL activity curve in human beings, dogs, and rabbits are shown in the Table I. The mean half-life of LPL activity in the circulation was 25.4 minutes in man, 18.6 minutes in dogs, and 7.3 minutes in rabbits. The half-life of injected heparin in the circulation was calculated to be 2.4 minutes in human subjects, 2.3 minutes in dogs, and 0.7 minutes in rabbits. The changes of heparin concentrations in the blood calculated from the curve analysis were parallel to those of coagulation time after heparin injection.

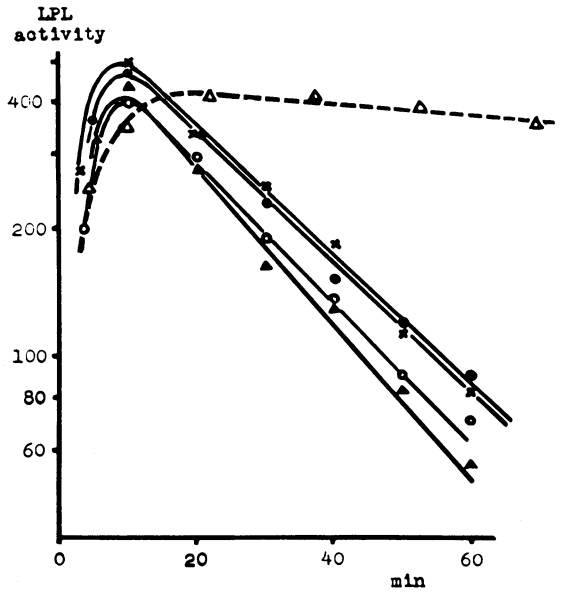

FIG. 2. THE CHANGES IN LPL ACTIVITY AFTER THE INJECTION OF HEPARIN, 0.1 MG PER KG OF BODY WEIGHT, IN NORMAL DOGS (SOLID LINES) AND A HEPATECTOMIZED DOG (DASHED LINE).

LPL activity curve in patients with liver diseases. Six cases of liver cirrhosis, four of acute hepatitis, one of hepatic cancer, and one of liver abscess were examined.

The results are shown in Table I. The *a*'s and *K*'s in Table I were calculated by using the average t_{max} value (time when the maximal LPL activity curve value was obtained) of the normal subjects, i.e., 9.1 minutes. Judged from curve analysis, this assumption may be reasonable.

The mean half-life of LPL in the circulation was 39.0 minutes in liver cirrhosis, which was significantly longer than that in normal subjects ($p < 0.01$).[2] In cases of acute hepatitis, except one, the half-lives were almost the same as that

[2] Student *t* test was used for statistical significance throughout the experiments.

of the normal subjects, and the mean value was not different from that of the control. In hepatic cancer and liver abscess, the half-life was identical with the normal value.

Constantinides, So, and Johnstone (3) and Baker, Levine, Turner, and Dubin (4) observed the higher LPL activity in the postheparin blood in liver cirrhosis and experimental liver damages and suggested that the ability to inactivate LPL was decreased in these hepatic disorders. Connor and Eckstein (5) confirmed these findings by assaying LPL activities in cannulated blood from the hepatic and systemic veins, the same as that of the portal vein in respect to LPL activity. The results of our experiment also support these findings.

In general, the rate of LPL inactivation in the liver may depend on the number of functioning liver cells, the function of each liver cell, hepatic blood flow, and the combinations of all or some of these.

In liver cirrhosis, the decrease in hepatic blood flow might play the most important role in the observed decrease in the rate of LPL inactivation.

In acute hepatitis, although hepatic blood flow had been reported to be decreased only slightly (6), the decreased inactivation of LPL was expected. In agreement with our results, however, Baker and associates (4) suggested that there was no decreased ability to inactivate LPL in patients with acute hepatitis. The reason for unexpected results, in which the rate of LPL inactivation in

TABLE I

Coefficient and exponential constants of lipoprotein lipase (LPL) activity curve in human subjects, dogs, and rabbits injected with heparin, 0.1 mg per kg of body weight

Diagnosis		K*	a†	b	t_{max}‡	$t_{\frac{1}{2}}$§
					min	*min*
Normal man		260	0.327	0.0235	8.7	29.5
		230	0.267	0.0263	9.8	26.4
		253	0.276	0.0263	9.5	26.4
		177	0.286	0.0320	8.7	21.6
		249	0.291	0.0263	9.1	26.4
		261	0.282	0.0244	9.5	28.3
		264	0.272	0.0281	9.3	24.7
		290	0.296	0.0350	8.2	19.8
	Mean	248	0.287	0.0277	9.1	25.4 ± 3.05‖
Liver cirrhosis		139	0.372	0.0143		48.5
		159	0.354	0.0164		42.4
		171	0.342	0.0180		39.1
		167	0.317	0.0217		31.8
		122	0.354	0.0164		42.3
		187	0.318	0.0231		30.1
	Mean	158	0.341	0.0183		39.0 ± 6.37‖
Acute hepatitis		88	0.333	0.0191		36.2
		129	0.268	0.0311		22.6
		104	0.284	0.0276		25.1
		127	0.260	0.0329		21.1
	Mean	112	0.286	0.0277		26.3
Hepatic cancer		120	0.284	0.0277		25.1
Liver abscess		178	0.283	0.0279		24.9

* $L = [K/(a - b)](e^{-bt} - e^{-at})$. For the explanation of the terms, see the text. The K $(=cH_0)$ was calculated by extrapolating each regression line fitted to the declining part of the curve. Then, the ordinate should equal $K/(a - b)$.

† The a's in the cases of hepatic disease were calculated from the equation, $t_{max} = (\log a - \log b)/[0.434(a - b)]$, with the mean t_{max} value of normal subjects 9.1 minutes.

‡ The t_{max} means the time when the maximum of the L value (LPL activity curve) was obtained. It was calculated as follows: $dL/dt = [K/(a - b)](ae^{-at} - be^{-bt}) = 0$. Therefore, $t_{max} = (\log a - \log b)/[0.434(a - b)]$.

§ The $t_{\frac{1}{2}}$ means the half-life of LPL.

‖ ± 1 SD of the mean.

¶ RES represents reticuloendothelial system, the blockade of which was attained by repeating injections of Chinese ink. (See the text.)

TABLE I—(*Continued*)

Diagnosis		K*	a+	b	t_{max}‡	$t_{\frac{1}{2}}$§
		149	0.238	0.0313	9.8	22.1
Obesity		356	0.331	0.0408	7.3	17.0
		255	0.239	0.0298	9.9	23.3
		259	0.231	0.0323	9.9	21.5
	Mean	255	0.260	0.0336	9.2	21.0 ± 2.38‖
		220	0.268	0.0256	9.7	27.1
		275	0.307	0.0336	8.1	20.6
Atherosclerosis		134	0.238	0.0351	9.5	19.8
				0.0360		19.3
		122	0.240	0.0382	9.1	18.1
	Mean	188	0.263	0.0337	9.1	21.0 ± 3.17‖
		196	0.314	0.035	7.9	19.8
		183	0.305	0.035	8.0	19.8
Normal dog		162	0.289	0.042	7.8	16.5
		143	0.273	0.038	8.5	18.3
	Mean	171	0.295	0.038	8.1	18.6
Hepatectomized dog				0.0027		256.7
		836	1.001	0.081	2.7	8.6
Normal rabbit		1,126	1.082	0.136	2.2	5.1
		888	0.624	0.085	3.7	8.2
	Mean	950	0.962	0.101	2.9	7.3
Hepatectomized rabbit				0.0060		115.5
				0.0096		72.2
		846	0.874	0.097	2.8	7.2
RES-blocked¶ rabbit		714	0.932	0.116	2.6	6.0
		983	0.705	0.114	3.1	6.1
	Mean	848	0.875	0.109	2.8	6.4

acute hepatitis was almost normal, is unknown. The destruction of liver cell membranes in acute hepatitis might promote contact of LPL with its inactivating enzyme in the liver cells, resulting in the acceleration of the degradation of LPL.

The peak values of the LPL curve were markedly lower in all cases of liver disease than in normal. This does not agree with the results of Constantinides and associates (3), Baker and co-workers, (4) and Sandhofer, Sailer, and Braunsteiner (8), but does agree with Connor and Eckstein (5) and Kern and Sanders (7).

Judged from the K's in Table I, the rate of release of LPL from the peripheral tissues (expressed by c) was markedly lower in patients with liver diseases, even when the half-life of LPL was normal, as in the cases of acute hepatitis. Sandhofer and co-workers (8) suggested also that the low LPL activity in patients with liver diseases might be due to the decreased release of LPL, but not to any inhibitor in the circulation.

Experimental studies on the role of the liver in the inactivation of LPL. Jeffries (9) and Spitzer and Spitzer (10) found that LPL was destroyed in the liver. But as mentioned above, unexpectedly in acute hepatitis the rate of inactivation of LPL seemed to be normal. Therefore, in order to examine the role of the liver in the inactivation of LPL, the following experiments were performed.

Functional hepatectomy was performed on a dog and two rabbits under Nembutal anesthesia by ligating portal vein, hepatic artery, and common bile duct together.

In the dog, the rate of LPL inactivation was only about 7% of that of normal dogs ($t_{\frac{1}{2}}$, 257 minutes; Figure 2). In rabbits, the average rate of LPL inactivation was only about 6% of that of normal rabbits ($t_{\frac{1}{2}}$, 94 minutes; Figure 3). The results may indicate that LPL is mostly inactivated in the liver.

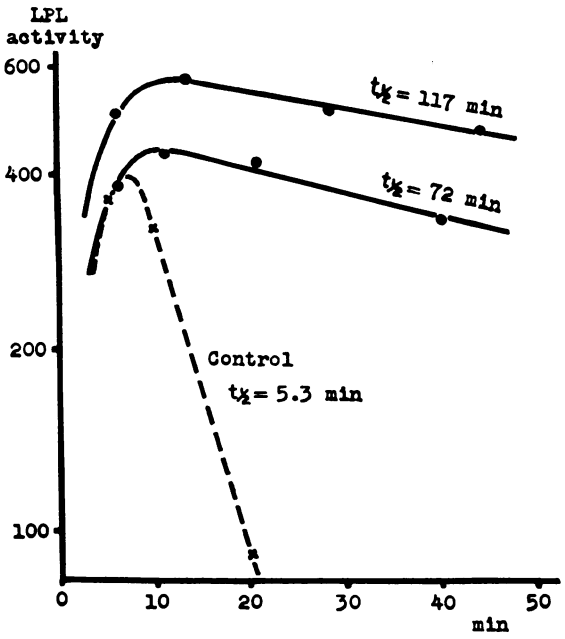

FIG. 3. THE CHANGES IN LPL ACTIVITY AFTER HEPARIN INJECTION, 0.1 MG PER KG OF BODY WEIGHT, IN HEPATECTOMIZED RABBITS.

Experimental studies on the site of inactivation of LPL in the liver. Blockade of the reticuloendothelial system was performed in rabbits by the daily injection for 5 days of 5 ml per 1 kg of body weight of Chinese ink [3] which was diluted 5 times with physiological saline solution.

Rabbits injected with Chinese ink usually lost appetite and body weight. Therefore, the control rabbits as well as the injected rabbits were fasted for the last 2 days.

On day 6, the rabbits were injected with heparin as mentioned in the Methods section, and LPL activity curves were obtained. Reticuloendothelial blockade did not influence the LPL activity curve in the circulation (Table I). The parenchymal cells, not Kupffer's cells, seemed, therefore, to inactivate LPL in the liver.

Mitochondrial fraction and supernatant fluid were obtained from mice livers by the method of Stein, Tietz, and Shapiro (11). The inhibitory effect of these fractions on LPL activity of heparinized human plasma was examined by preincubating 1 ml of heparinized plasma with 1 ml of each fraction and then determining residual LPL activity by the above-mentioned method. The mitochondrial fraction showed about 3 times greater degree of inhibition than the supernatant fluid.

By treating animals either with allyl formate (periportal fatty degeneration) or with carbon tetrachloride (centrolobular), the inhibitory effects of the liver homogenates were reduced in all fractions to about 75% of those of the normal liver homogenates (Table II).

LPL activity curve in obesity. The LPL activity curves were determined in four cases of obesity without any apparent clinical diseases (Broca's index: 1.20 to 2.03). The half-life of LPL was 21.0 ± 2.38 minutes, which was significantly shorter than that of the healthy control, 25.4 ± 3.05 minutes ($0.01 < p < 0.05$) (Table I).

The authors could only find a report on LPL in obese subjects by Sandhofer and associates (8). They observed a close linear relationship between body weight and LPL activity in 75 cases ($0.05 > p > 0.02$). Our study did not confirm their results; the peak values were the same in both obese and normal subjects in our experiment.

It is unknown whether the increased ability of inactivating LPL is concerned with the development of obesity.

LPL activity curve in atherosclerotic patients. The LPL activity curves were determined in five patients with coronary sclerosis. The half-life of LPL was 21.0 ± 3.17 minutes, with a significant difference from the control group ($0.02 < p < 0.05$) (Table I).

Heparin as well as heparin-induced LPL has

TABLE II

Inhibitory effect of the liver homogenates of mice on lipoprotein lipase (LPL) activity

	Inhibition of LPL activity		
	Normal liver	CCl_4-injured liver*	Allyl formate-injured liver*
	%/100 μg of N		
Full homogenate	21.8	17.9	18.5
Supernatant fluid	15.3	11.5	12.3
Mitochondria	42.4	31.5	32.4

* A volume of 0.2 ml of 10% carbon tetrachloride or 0.25 ml of 2% allyl formate was subcutaneously injected once in each male mouse (around 25 g of the body weight). The mice were sacrificed 48 hours after the injection. The figures are the average of three duplicate experiments.

[3] Kaimei Bokujyu, Taguchi Co., Ltd., Tokyo, Japan.

been intensively studied by many investigators as related to atherosclerosis. Some (12–17) observed lower LPL activity in patients with atherosclerosis, but others (8, 18–22) found no difference in LPL activity between normal and atherosclerotic subjects. Our results showed that the peak values were the same in both groups.

Whether the observed increase in the rate of inactivation of LPL is concerned with the occurrence of atherosclerosis remains to be solved.

CONCLUSION

The levels of lipoprotein lipase (LPL) activity were followed for a period of time after heparin injection, and the metabolism of LPL in the circulating blood was studied from the dynamic point of view. The results were as follows:

1) The LPL activity rose rapidly to reach the peak at about 9 minutes in man, about 8 minutes in dogs, and about 3 minutes in rabbits. The LPL activity curve was expressed by the difference of 2 exponential terms, $L = [cH_0/(a - b)](e^{-bt} - e^{-at})$. (For the explanation of the terms, see the text.)

2) The mean half-life of LPL in the circulation was 25.4 minutes in man, 18.6 minutes in dogs, and 7.3 minutes in rabbits.

3) The average of calculated half-life of injected heparin was 2.4 minutes in man, 2.3 minutes in dogs, and 0.7 minutes in rabbits.

4) The mean half-life of LPL activity in patients with liver cirrhosis was significantly longer than the normal value, while that in acute hepatitis, liver abscess, and liver cancer was normal. Calculated production rate of LPL was lower in liver diseases.

5) Functional hepatectomy in a dog and rabbits inhibited almost completely the inactivation of LPL in the circulation.

6) The blockade of the reticuloendothelial system by Chinese ink did not influence the LPL activity curve in the circulation. There was 3 times higher inhibitory activity on LPL in mitochondria than in the supernatant fluid of the mouse liver.

7) The rate of LPL inactivation in the circulation was significantly shorter in obesity and in coronary sclerosis.

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