LIPOPROTEIN LIPASE AND LECITHIN-CHOLESTEROL ACYLTRANSFERASE ACTIVITY IN EXPERIMENTALLY MODIFIED LIPOPROTEIN METABOLISM

M. G. Tvorogova, V. N. Titov, and N. V. Perova

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Lipoprotein lipase (LPL) and lecithin-cholesterol acyltransferase (LCAT) are the principal enzymes of lipoprotein (LP) transformation in the vascular system. LPL, localized on the surface of the capillary endothelium, catalyzes hydrolysis of triglycerides of very low density lipoproteins (VLDL) and chylomicrons (ChM). Injection of heparin causes release of LPL into the blood stream and enables activity of the enzyme in the blood plasma to be measured, hence the term "postheparin lipoprotein lipase" [3]. LCAT, an enzyme synthesized in the liver and circulating in the blood plasma, catalyzes the conversion of lecthin and of free cholesterol into lysolecithin and cholesterol (CH) esters respectively. This reaction is not only the principal mechanism of formation of CH esters in the blood plasma, but it also plays an important role in LP metabolism, directly or indirectly affecting the lipid and protein composition of each class of LP [9].

The foundations of theoretical research into interconnection between the principal enzymes of LP tranformation, namely LPL and LCAT, were laid by the hypothesis of combined of LPL and LCAT in VLDL catabolism [14]. According to this hypothesis, removal of triglycerides from the hydrophobic nucleus of the lipoprotein, brought about by LPL, ought to be accompanied by a decrease in the hydrophilic surface of the particle, which takes place through the participation of LCAT, by the action of which CH and lecithin are removed in equimolar proportions from the particle surface. However, in subsequent experiments [8] it was conclusively proved that LCAT esterifies CH only in high density lipoproteins (HDL). Other investigations [11] revealed that HDL *in vitro* do not stimulate LCAT activity, whereas LP enriched with tryglycerides, have an activating effect on esterification of CH in the blood. The contradictions between these data indicate that the role of LCAT in the process of VLDL catabolism requires further study.

On the basis of the hypothesis of the simultaneous participation of LPL and LCAT in VLDL catabolism [14] it may be expected that activity of these enzymes would change in the same direction of different physiological states. In fact, an increase in activity of both LPL [12] and LCAT [15] has been observed in a state of alimentary lipemia. However, LCAT activity was found to be increased in types of hyperlipoproteinemia that characterized by hypertriglyceridemia and fall in LPL activity [1, 15].

In the present investigation LPL and LCAT were studied in experiments on rats with modified lipid metabolism under the influence of certain drugs. The aim of the investigation was to study relations between serum LPL and LCAT activity under normal conditions and after various changes in lipid metabolism.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-200 g. Altogether 120 rats were used in the experiments. They were kept in a room with standard lighting conditions — from 8 a.m. to 8 p.m. For 2 weeks before the experiment and during its course the rats received dry pellet food ad lib. Three types of modified LP metabolism were used.

In the experiments of series I the animals were given clofibrate (Miscleron, from Egyt, Hungary) in a dose equivalent to 0.5% of the weight of the blood (about 100 mg daily) for 7 days.

All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.
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TABLE 1. Changes in LPL Activity of Peripheral Tissues and LCAT Activity in Blood Serum of Rats under the Influence of Drugs (M ± m, n = 10)

Drug	LPL activity, nmoles/ml·min		LCAT activity, moles/ml·h	
	Control	Experiment	Control	Experiment
Clofibrate	100,8±9,1	123,4±9,10*	11,9±0,9	12,6±1,0
Cholestyramine	107,4±3,8	105,8±4,3	10,9±0,7	18,4±1,8*
Ethinyl-estradiol	73,6±1,5	56,8±4,6*	14,5±0,4	9,7±1,7*

Legend. *P < 0.05 compared with control.

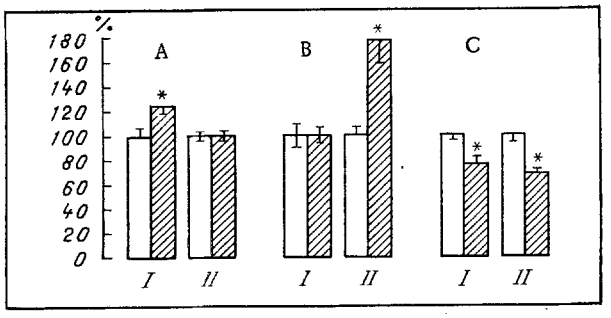

Fig. 1. Comparison of LPL (I) and LCAT (II) activity in different types of modified LP metabolism. A) clofibrate, B) cholestyramine, C) ethinyl estradiol. Unshaded columns — control, shaded — experiment. Control values taken as 100%. *P < 0.05.

In series II the rats were given cholestyramine (Questran, from Mead Division of Myers Company, England) in a dose of 2% of the weight of the food (about 400 mg daily) for 7 days. In series III the rats were given ethinyl-estradiol intramuscularly in a dose of 50 μg/kg in 0.2 ml of olive oil daily for 5 days. Rats of the control group received 0.2 ml of olive oil daily.

According to previous data [2], these doses and duration of administration of clofibrate, cholestyramine, and ethinyl-estradiol give rise to considerable changes in lipid metabolism in rats.

Incidentally, it is inadvisable to determine LPL and LCAT activity in the same sample of blood serum, because this does not reflect the ratio between their activities under physiological conditions. Injection of heparin, necessary when determining LPL activity in the blood stream, at the same time inhibits LCAT activity [13]. This phenomenon is due to competition between free fatty acids (FFA; products of the LPL reaction) and lysolecithin (product of the LCAT reaction) for binding sites in the albumin molecule, which has higher affinity for FFA. Saturation of the binding sites in the albumin molecule leads to inhibition of the LCAT reaction by excess of the reaction end product. To prevent this effect, in each series of experiments to create model of modified LP metabolism, both the experimental (receiving the prepration) and the control animals were randomized into two groups for determination of LPL and LCAT separately.

To determine LPL activity, rats of the control and experimental groups were given an intraperitoneal injection of heparin (from Gedeon Richter, Hungary) in a dose of 100 U/kg 15 min before decapitation. LPL activity of the peripheral tissues and triglyceride lipase activity of the liver, simultaneous release of which into the blood stream takes place under the influence of heparin, were determined by a radiologic method [1]. LPL activity of the peripheral tissues was calculated as the difference between the percentage hydrolysis of glycerol tri(1-^{14}C)-oleate before and after incubation of the postheparin plasma with protamine sulfate, an inhibitor of LPL activity [1]. LCAT activity was determined by a radiologic

method [7] in animals not receiving heparin.

EXPERIMENTAL RESULTS

To rule out the effect of seasonal fluctuations in blood lipid levels of the experimental animals [4] on the experimental results, in each series, besides the 10 experimental (receiving the drug) animals, parallel tests also were carried out on 10 control rats. The data on the change in LPL and LCAT activity in the three different models modified LP metabolism are given in Table 1. Differences between activity of the enzymes in animals of the control groups for the experiments with colfibrate and cholestyramine were not statistically significant from one series to the next. Changes in enzyme activity in animals of the control group in the series of experiments with ethinol-estradiol compared with those in the series mentioned above were evidently attributable to the effect of the daily injections of olive oil.

Comparison of activity of the enzymes on the three different experimental models of modified LP metabolism showed that the use of only one of them — that with ethinyl-estradiol — a change in activity of both enzymes, LPL and LCAT, in the same direction (a decrease). Under the influence of clofibrate LPL activity increased whereas LCAT activity was unchanged; cholestyramine caused no change in LPL activity, but led to an increase in LCAT activity (Fig. 1). These experimental results showed that correlation between activity of the main enzymes of LP transformation in the vascular system varies.

On the basis of these experimental results and of data in the literature [5, 6, 10] the question of relations between LPL and LCAT can be expressed in a rather different form from that adopted previously [14]: the substrate for the enzyme consists of lipids that are components of the same triglyceride-enriched LP, but the triglycerides are hydrolyzed by LPL while directly in the composition of these LP, and the free CH is esterified in the composition of surface fragments removed from LP, and similar to the "nascent" HDL, the principal substrate for LCAT, or on the surface of complexes consisting of the following components: the fragments of particles of HDL mentioned above, the sole protein of which is apo-A-1, the enzyme LCAT, and apo-D. In addition, the free CH of the cell membranes, to be accepted by HDL, also undergoes esterification in these complexes through the action of LCAT. The LCAT reaction is thus a universal process aimed at maintaining the physiological level of CH and its esters in the blood plasma.

It can thus be tentatively suggested that synergism in changes in LPL and LCAT activity exists only when activation of lipolysis of triglyceride-saturated LP leads to the formation of many particles consisting of secondary remnants of VLDL, which are fragments of their surface layer and are similar in composition and properties to "nascent" HDL, the principal substrate for LCAT.

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