LIPOPROTEIN LIPASE AS HUMORAL AGENT OF THE PHYSIOLOGICAL ANTICLOTTING SYSTEM

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The formation of thrombin in the circulating blood in a concentration presenting a risk to life may result from a number of causes, including alimentary lipemia. The appearance of lipoprotein particles (chylomicrons) of low density in the blood may provoke thrombinogenesis as a result of the thromboplastic activity inherent in these particles [36, 37, 40]. The thromboplastic activity of the chylomicrons is due to the fact that they contain certain phospholipids which stimulate and accelerate the process of blood clotting [15, 32], like tissue thromboplastin.

The appearance of thrombin in the blood as a result of alimentary lipemia may evidently cause a vicious circle: thrombin blocks the activity of lipoprotein lipase [39], leading to prolonged retention in the plasma of lipoprotein particles, causing thrombinogenesis. However, the appearance of a slight excess of thrombin in the circulating blood of the healthy organism does not lead to thrombosis because of the protective reaction of the physiological anticlotting system [3-8]. This reaction takes the form of a reflex secretion of various humoral agents into the circulating blood, preventing clotting and neutralizing thrombin. Among these substances are heparin and the heparin-like substances [2, 8, 10]. An increase in the concentration of heparin in the circulating blood has also been demonstrated after the intravenous infusion of a fat emulsion [19]. It was shown as far back as 1943 [30] that heparin in vivo accelerates and stimulates the clearing of plasma in alimentary lipemia by activation of lipoprotein lipase [17, 18, 21, 22, 33, 34, 35]. Because of these findings, lipoprotein lipase was considered to be a component of the physiological anticlotting system [5, 9, 12]. In experiments on rats reared on Wilgram's diet [42, 43], we found [1, 10] that this type of atherogenic diet leads to depression of the function of the physiological anticlotting system after 5-8 months of the experiment, as a result of which an acute prethrombotic state develops, with certain definite diagnostic signs. In these conditions, the animals often showed spontaneous conversion of the prethrombosis into a thrombosis, which we were inclined to attribute to provocation of thrombin formation in the circulating blood because of the combination of the persistent alimentary lipemia and depression of the physiological anticlotting system.

The object of the present research was to examine the importance of the lipoprotein lipase to the function of the physiological anticlotting system.

EXPERIMENTAL METHOD

Experiments were conducted on 450 male albino rats weighing 220-320 g. Normal control animals were kept on a balanced laboratory diet, while the experimental animals received Wilgram's atherogenic diet for a period of 8 mo. Blood for investigation was taken from the jugular vein of the rats by syringe. The heparin or thrombin solutions used in the experiments were given in the same way.

The fibrinolytic activity of the blood plasma and the fibrinogen concentration were determined by Bidwell's method [13]. The plasma heparin tolerance was obtained by Gormsen's method [23], and the heparin concentration in the plasma was calculated from the protamine sulfate titer by the method of Le Roy and co-workers [44]. The total blood cholesterol was determined by Grigant's method [27]. The lipoprotein lipase activity was judged by the velocity of hydrolysis of a standard triolein substrate (commercial preparation), used in a concentration of 300 or 500 mg% [29]. Free fatty acids were titrated with a 0.01N KOH solution. The results were expressed in mg% in terms of oleic acid with a molecular weight of 282. The method of determining the fatty acids was taken from the papers by Borgström and co-workers [14], Engelberg [18], and Grossman [28].

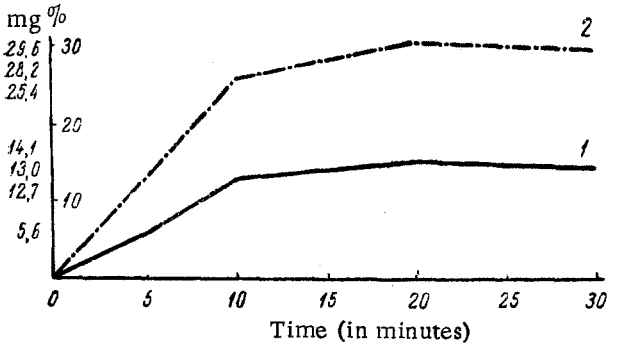

Fig. 1. Lipoprotein lipase activity (in mg% of free fatty acids) of the blood plasma of 36 experimental rats (1) in a prethrombotic state caused by an atherogenic high-fat diet (for 8 months) and of 36 controls (2). Mean values.

Plasma Heparin Concentration in Rats in a Prethrombotic State Caused by an Atherogenic Diet (for 8 months)

Rat No.	Experimental	Rat No.	Control
36	40	2	100
86	20	1 и 49	100—120
88	30	35 и 45	100—120
90	50	36 и 48	100—120
101	40	37 и 46	100—120
106	30	—	—
107	20—30	—	—
116	20—30	—	—
Mean	32,5		110

Note. The figures (experiment and control) denote the weight of protamine sulfate (in μg) neutralized by 30 μg of pure heparin added to 1 ml of plasma.

EXPERIMENTAL RESULTS

After the experimental rats had received Wilgram's diet for 8 months, they were in an acute prethrombotic state, characterized by an increase in the fibrinogen concentration on the average to 628.5 mg% (control 348.5 mg%) and a sharp fall in the fibrinolytic activity of the blood on the average to 7% (control 38.6%), together with an increase in the plasma heparin tolerance (mean values, experiment 4 min, control 15 min, 13 sec). The total blood cholesterol was increased ten-fold: mean values, experiment 750 mg%, control 75 mg%.

All the rats in a prethrombotic state showed a marked decrease in the plasma lipoprotein lipase activity (Fig. 1). In the experiments undertaken to study the causes of this phenomenon, the blood heparin concentration of the experimental animals was found to be lowered to half or one-third the control value (see table).

The intravenous injection of 50, 75, 100, and 200 units of heparin into an experimental rat led to a sharp increase in the lipoprotein lipase activity (Fig. 2). It should be noted, however, that the activity of this enzyme in the blood of the experimental animals never reached the level characteristic of the plasma of the control rats receiving identical doses of heparin.

These results show that the lipoprotein lipase activity of animals in a state of prethrombosis caused by Wilgram's atherogenic diet was depressed as a result of heparin deficiency. In view of the fact that after intravenous injection of identical doses of heparin into the animals of the control and experimental groups, the lipase activity in the latter did not reach its level in normal rats, it may be postulated that besides the heparin deficiency the animals in a prethrombotic state also suffered from a relative deficiency of the enzyme itself.

When normal animals received intravenous injections of thrombin solution in doses of 0.8-1.2 ml (from 12 to 18 Mellamby units) their lipoprotein lipase activity showed a tendency to increase (Fig. 3).

Thrombin, when taken in equivalent amounts, meanwhile inhibited the plasma lipoprotein activity in vitro, lowering it by almost half. The intravenous injection of thrombin in doses of 0.5-0.6 ml (activity 7.5-9 units) caused no perceptible increase in the lipoprotein lipase activity, although titration of the heparin in the plasma of these animals with protamine sulfate revealed a relative excess of this substance. For instance, before intravenous injection of thrombin, the neutralization of 30 μg heparin added to 1 ml of plasma required on the average 70-80 μg of protamine sulfate. Meanwhile, in the same conditions but 4-5 min after intravenous injection of thrombin, 100-120 μg of protamine sulfate was required to neutralize the same quantitiy of heparin.

The activation of lipoprotein lipase after the intravenous injection of thrombin into the animals was due to the appearance of heparin in the blood stream as a result of a reflex humoral mechanism operated by the protective anticlotting system. The tendency for the lipoprotein lipase activity to increase was maximal 5 min after injection of thrombin or after incubation of the plasma for the same period. It seems that the heparin released into the blood stream remains in the free state for only a short time, after which it forms a complex with proteins and thereby loses its ability to activate lipoprotein lipase.

Our results demonstrate that the lipoprotein lipase of the plasma is associated with the physiological anticlotting system: heparin secreted by a reflex mechanism into the blood stream activates the enzyme and thereby accelerates

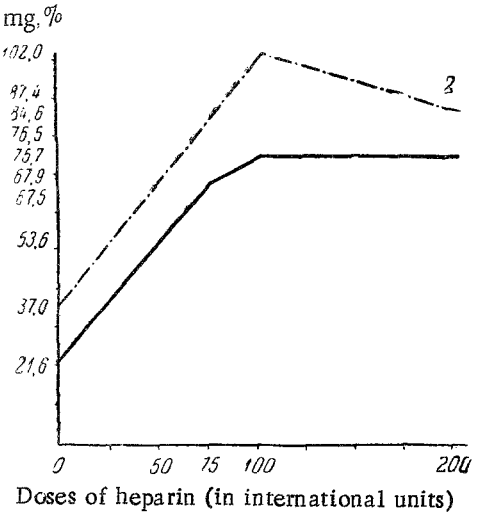

Fig. 2

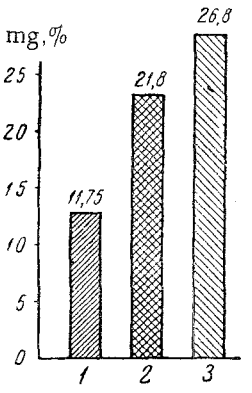

Fig. 3

Fig. 2. Plasma lipoprotein lipase activity of 170 control rats (2) and of 60 rats in a prethrombotic state caused by atherogenic high-fat diet (1), depending on the intravenous injection of different doses of heparin. Mean values.

Fig. 3. Change in lipoprotein lipase activity (in mg% of free fatty acids) in 140 normal animals after addition of thrombin in vitro (1), and before (2) and after (3) injection of thrombin in vivo. Mean values.

the clearing of the plasma in alimentary lipemia . This physiological reaction in the healthy organism is partially suppressed or absent in the prethrombotic state,caused by prolonged feeding with an atherogenic diet.

We also know that alimentary lipemia depresses fibrinolysis [1, 11, 16, 20, 24, 25, 26, 31, 41]. The prolonged survival of lipoprotein particles in the plasma of animals kept on an atherogenic diet [1, 11] and in human patients with atherosclerosis [38] is evidence of a disturbance of the activation of lipoprotein lipase by the physiological anti-clotting system, and of the creation of conditions in the organism particularly favorable for the development of atherosclerosis and of intravascular thrombosis.

SUMMARY

Experiments were staged on 450 male albino rats. As established, a stable food lipemia was attained after keeping the animals on a fat diet(according to Wilgram [42,43]) for 8 months. The activity of lipoprotein plasma lipase in such animals was half as compared to control animals which were given the usual laboratory ration. Intravenous injection of heparin led to considerable activation of lipoprotein lipase in experimental animals. However, it did not reach the level attained in control animals after injection of the same doses of heparin. Intravenous injection of thrombin to normal animals led to activation of lipoprotein lipase due to the appearance in the blood of some excess of reflex-secreten heparin by the physiological anticoagulating system [4, 7, 10] in response to the appearance of thrombin in the circulation.

Thus, the development of prethrombotic state in animals kept on a fat diet was accompanied by reduction of lipoprotein lipase activity due to deficiency of heparin and partially of the enzyme itself. A conclusion was drawn that lipoprotein lipase was connected with the function of the physiological anticoagulating system.

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