LIPOPROTEIN LIPASE FROM HEART AND LIVER: AN IMMUNOLOGICAL STUDY

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SUMMARY: Lipoprotein lipase activity was purified from rat postheparin serum. An antibody against this activity was raised. It was found that this antibody inhibits 95% of the postheparin lipoprotein lipase activity. The same inhibition was found in the perfusate of heparin-perfused rat liver. When measuring lipoprotein lipase activity in perfusates of rat hearts, perfused with heparin, no inhibition by this antibody was found. It is concluded that lipoprotein lipase activities from heart and liver are catalysed by different enzymes and that the heart enzyme contributes not more than 5% to the overall lipase activity in postheparin serum.

Korn (1) showed in 1955 that acetone powders of rat heart contained lipoprotein lipase. This enzyme is known also to be localized in lung, adipose tissue, diaphragm and mammary gland. After intravenous injection of heparin, lipoprotein lipase activity appears in the blood (2). This activity is assumed to be released from extrahepatic organs. However a number of authors (3,4,5) have shown recently that lipase activity is also released from the liver. Borensztajn et al. (6) had found that a large part of the original lipoprotein lipase activity was removed from the heart after perfusion of rat heart with a heparin containing medium. Liver shows a very rapid release of lipase activity on perfusion with heparin. Therefore we investigated the contributions of liver and heart to the lipase activity of postheparin plasma.

METHODS

Rats were injected with heparin (100 I.U./kg bodyweight). After 6-15 min they were killed and the blood was collected. Lipoprotein lipase activity was purified from the serum on a sepharose-heparin co-

lumn, as described for lipoprotein lipase from skim milk by Olivecrona and Egelrud (7). We replaced the NaCl gradient by a heparin gradient (0-1.25 mg/ml). The purification was 600-fold. In a subsequent calciumphosphate adsorption step another two-fold purification was obtained. 1 mg of the purified enzyme, mixed with Freunds adjuvant (1:1), was injected in the foot pads of a rabbit. After 4 weeks a booster injection was given intramuscularly and 10 days later 30 ml blood was collected by heart punction. Purification of the γ-globulin fraction was achieved by ammonium sulphate precipitation (50% saturation) and gel filtration on a Sephadex G-200 column, followed by another ammonium sulphate precipitation. Electrophoresis (not shown) indicated that the isolated fraction contained only γ-globulins. Serum of a non-immunised rabbit was handled in the same way. This γ-globulin fraction was used in control experiments.

Rat hearts were perfused, after Nembutal narcose of the animals, according to the Langendorff technique with a modified Tyrode solution for 10 min at 37°C. Then the perfusion medium was replaced by one containing, in addition, 20% (v/v) preheparin rat serum and 4.5 I.U. heparin/ml.

Rat livers (about 8 g) were perfused through the portal vein with a Krebs-Henseleit bicarbonate buffer, saturated with 95% O_2 - 5% CO_2, containing 5 mM glucose at 33 ± 2°C. The inferior caval vein was ligated below the liver and cannulated above the liver. After 10 min perfusion at a rate of 20 ml/min the perfusion medium was replaced by a medium, containing 80% of the medium described above and 20% of rat serum (v/v) as well as heparin (final concentration 4.5 I.U./ml). The top of the latter was briefly sprayed with Antifoam (Dow Corning Corp.).

Lipoprotein lipase activity was estimated with palmitoyl-CoA as the substrate, as described by Jansen and Hülsmann (8). When [^{14}C]trioleate was used as the substrate, the modified method of Kelly, as descri-

Table I

INFLUENCE OF PREHEPARIN SERUM ON PURIFIED AND ANTIBODY TREATED LIPOPROTEIN LIPASE

Enzyme source	Preincubation*	Additions to standard assay	Activity
Purified enzyme	none	none	1200 mU/mg
" "	none	10 µl human serum	1865 mU/mg
" "	none	10 µl rat serum	1880 mU/mg
Postheparin serum	60 min, no additions	none	140 mU/ml
" "	60 min, 15 µl control γ-glob. fraction present	none	136 mU/ml
" "	60 min, 15 µl control γ-glob. fraction present	10 µl human serum	148 mU/ml
" "	60 min, 15 µl control γ-glob. fraction + 10 µl human serum present	none	143 mU/ml
" "	60 min, 15 µl antibody fraction present	none	7 mU/ml
" "	60 min, 15 µl antibody fraction present	10 µl human serum	6 mU/ml
" "	60 min, 15 µl antibody fraction + 10 µl human serum present	none	8 mU/ml

Incubations were carried out as described in Fig. 1. The blood was withdrawn 6 min after heparin injection and centrifuged; the plasma was tested. Palmitoyl-CoA was the substrate.

*Where shown, incubation of 5 µl postheparin serum was for 60 min at 37°C, followed by centrifugation for 2 min at 15 000 x g.

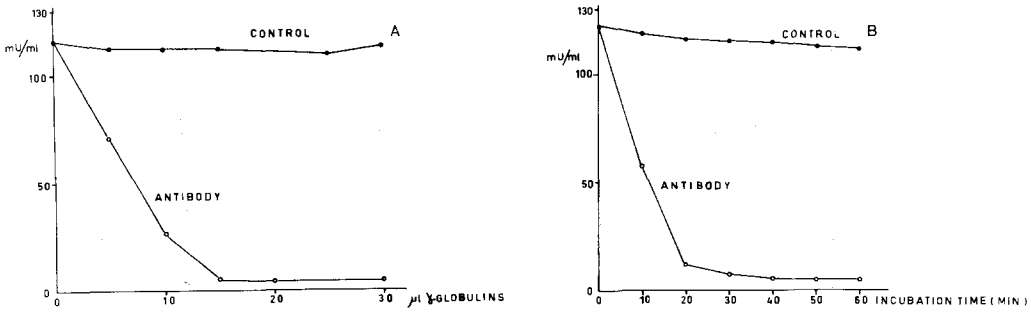

Fig. 1. Influence of different amounts of antibody and different incubation times on postheparin lipoprotein lipase activity.

20 µl postheparin plasma was either incubated for 1 h with different amounts of antibody- or control preparation at 37°C (Fig. 1A) or at different incubation times, as shown in Fig. 1B. The mixtures were centrifuged for 2 min at 15 000 x g. From the supernatants amounts equivalent to 5 µl original postheparin serum were tested. The amounts of control- or antibody preparation used were related to 5 µl postheparin serum.

bed in ref. 8, was used. Activities are expressed as nmoles CoASH released per min in the palmitoyl-CoA assay, and as nmoles FFA released/min, when trioleate was the substrate.

RESULTS

The antibody preparation was diluted with 0.9% NaCl to a protein concentration of 3.35 mg/ml, the same was done with the control serum fraction. By varying the antibody concentration and the incubation time we found that 15 µl antibody inhibited lipoprotein lipase activity of 5 µl whole postheparin serum 95%, when incubated between 30 and 60 min at 37°C, followed by centrifugation for 2 min at 15 000 x g (Fig. 1A and B). The presented values are based on linear reaction rates measured during the first 10 min. In order to exclude that an enzyme labile at 37°C was inactivated during the incubation with control or antibody, we incubated postheparin serum with a non-saturating amount of control and antibody at 0°, 37° and 40°C. It was found that during the incubation at 0°C for 3.5 h no activity was lost and that

with this amount of antibody 70% of the initial activity was inhibited. Incubation at 37°C gave a loss of 19% of the initial activity; of the remaining activity 70% was again inhibited by the antibody. Incubation for 1 h at 40°C with control γ-globulins resulted in 75% inactivation of the enzyme. The remaining activity was again 70% inhibited by the antibody. Therefore the existence of a heat-labile non-inhibited enzyme could be excluded.

In order to exclude that the inhibition resulted from antibody against activating proteins, we incubated antiserum together with preheparin human serum (preheparin human serum showed no precipitation lines in the Ouchterlony immuno diffusion assay with the antiserum) and added human serum to the inhibited enzyme. Table I shows that the addition of human serum before or after incubation with antiserum does not influence the reaction rate. It is also shown that the partially purified enzyme is activated in the same way by human serum as by rat serum. In another experiment (not shown) whole antiserum was mixed with preheparin rat serum and the resulting precipitate removed by centrifugation. From the supernatant, γ-globulins were purified as described before. It was found that with this preparation the same results were obtained as with the original γ-globulin fraction. From this and from Table I it can be concluded that the antibody inhibits the enzyme directly and that the inhibition is not caused by precipitation of activating plasma proteins.

In order to detect the principal source of lipase activity in postheparin serum, liver and heart were perfused in vitro with a heparin containing medium (Table II). It was found that lipoprotein lipase activity released from rat heart did not interact with the antibody, but that the liver perfusate is inhibited to the same extent as the postheparin serum lipase activity. After incubation for a longer time (60 min 37°C) the activity of the liver perfusate is completely inhi-

Table II

INHIBITION OF POSTHEPARIN SERUM-, HEART- AND LIVER LIPASE ACTIVITIES BY ANTIBODY AGAINST POSTHEPARIN SERUM LIPASE

Enzyme source	Pretreatment with		Lipoprotein lipase activity substrate used		% Inhibition substrate used	
	control	antibody	palmitoyl-CoA (mU/ml)	trioleate (nmoles/min/ml)	palmitoyl-CoA	trioleate
Postheparin serum	+	-	148	318		
" "	-	+	21	68	86	78
Heart perfusate	+	-	7.4	3.7		
" "	-	+	7.4	3.7	0	0
Liver perfusate	+	-	114	29.0		
" "	-	+	19	4.5	83	84

Liver and hearts were perfused as described. Fractions of 10 ml were collected. Most of the activity in the liver was released in the first 2 min (=40 ml). The heart released its activity more gradually; during 6 min (=60 ml) equal activities were obtained. Pretreatment of the perfusates was as described in Fig. 1. Incubations were done for 30 min at 37°C. 5 µl postheparin serum was incubated with 15 µl antiserum. Heart-and liver perfusates were incubated with equal volumes of anti- or control serum.

bited (not shown), whereas the lipase activity of postheparin serum is inhibited 95% (Fig. 1B).

DISCUSSION

From the data obtained it can be concluded that lipase activities that are released by heparin from heart and liver are catalysed by immunologically different enzymes. The lipase activity in serum, after heparin injection, behaves very similarly to the liver enzyme in response to the inhibitory action of the antibody (Table II). In this table it can be seen that the inhibition of postheparin serum lipase by the antibody, when measured with trioleate as the substrate, is 8% less than when palmitoyl-CoA is the substrate. This can be explained by the higher ratio of palmitoyl-CoA hydrolase activity over trioleate hydrolase activity in liver perfusate, when compared with heart perfusate. The data obtained in Table I allow the conclusion that the heart contributes maximally 5% to the lipase activity in postheparin plasma, when palmitoyl-CoA is the substrate, and not more than 13% when trioleate is the substrate. Kraus *et al.* (5) found, that about 65% of the lipase activity in postheparin serum was derived from the liver. It is tempting to conclude from our results that even up to 95% (87% with trioleate as the substrate) of the lipase activity is released by the liver. It is possible that we find a somewhat higher contribution by the liver because we used a smaller amount of heparin [100 I.U. vs. 250 I.U./kg body weight used by Kraus *et al.* (5)].

Another explanation would be that, in addition to heart and liver, other organs contribute to the serum activity and that this activity would be equally inhibited by the antibody. This is presently under investigation. It has already been found, that the purified postheparin serum enzyme showed only one protein band with hydrolase activity in polyacrylamide gel electrophoresis (pH 8.9) (not shown). Fielding (4) found that the liver enzyme had a different substrate specificity

than the activity released from other organs. The present paper shows, that this difference (at least for heart and liver) is due to different enzymes and not only to the influence of cofactors, as was discussed before by LaRosa et al. (3) and Kraus et al. (5).

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