ON THE ORGAN SPECIFICITY OF NEUTRAL GLYCEROLESTER HYDROLASE OF VARIOUS TISSUES; AN IMMUNOLOGICAL STUDY

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SUMMARY. Rat liver microsomal glycerol monoester hydrolase (EC 3.1.1.23) has been purified 130 fold. The enzyme has a molecular weight of about 60,000. An antibody raised against this enzyme in rabbit did not inhibit heparin-releasable liver lipase, which hydrolyses long-chain 1- and 2-monoglycerides effectively. This confirms an earlier conclusion, based on results obtained with an antibody raised against the latter enzyme, that the non-releasable and heparin-releasable liver enzymes are different proteins. The antibody against the liver microsomal glycerol monoester hydrolase, however, inhibited also the monoglyceridase activities of acetone powder extracts of rat small intestinal epithelial microsomes and rat epididymal fat pads, suggesting structural similarities between the endoplasmic reticulum hydrolases of various tissues. These findings also apply to pig where an antibody against adipose tissue lipases inhibits the monoglyceridase activities of small intestinal and liver microsomal acetone powder extracts.

More than two decades ago (comp. ref. 1), it was found that in a number of species heparin injection causes the release of two distinct glycerolester hydrolase activities. These are probably identical with (extrahepatic*-) lipoprotein lipase (EC 3.1.1.3) and hepatic postheparin lipase. The two enzymes are immunoglocigally different[2,3], have different substrate specificity[4,5] and may be inhibited differentially[1,6,7]. Whereas lipoprotein lipase preferentially hydrolyzes long-chain triacylglycerol esters, when compared with monoacylglycerol esters, the liver enzyme preferentially hydrolyzes monoglycerides[4,5]. This is reflected by the molar ratio of the fatty acids and glycerol released during triglyceride hydrolysis which exceeds 8 when lipoprotein lipase is the catalyst (due to

*) During perfusion of isolated rat liver with heparin not only salt resistent liver lipase is released but also salt sensitive, and relative labile, lipoprotein lipase. Up to one fifth of the total trioleoylglycerol hydrolyzing activity of a freshly obtained perfusate is inhibited by 1 M NaCl (unpublished).

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accumulation of 2-monoglycerides) and is 3 with the liver enzyme. The function of the heparin-releasable liver enzyme therefore may not only be triglyceride hydrolysis, when plasma levels are high, but mainly monoglyceride hydrolysis for which the apparent K_m is much lower and the V_{max} much higher[5] than for triglyceride hydrolysis.

A glycerol monoester hydrolase non-releasable by heparin that has no activity with long-chain di- and triacylglycerol esters, in contrast to long-chain monoacylglycerol esters, has also been described. This activity is present in a number of tissues, such as small intestinal epithelium[9,10], liver[5,11,12] and adipose tissue[13,14]. It was the purpose of the presented work to obtain more information on this (these) non-releasable microsomal enzyme(s) and to eliminate the possibility that in liver this enzyme is a precursor of the heparin-releasable lipase. Therefore, our immunological studies[2,3,5] were extended.

METHODS

Purification of rat liver microsomal glycerol monoester hydrolase

In order to remove heparin-releasable liver lipase, rat liver was *in vitro* perfused[3] for 20 min with 5 I.U. heparin per ml Krebs-Ringer bicarbonate buffer prior to homogenization in 0.25 M cold sucrose, containing 10 mM Tris-HCl (pH 7.4) and 1 mM ethylenediamine tetraacetate. After removal of nuclei by centrifugation for 5 min at 800 x *g*, mitochondria were removed at 8700 x *g* (10 min) and microsomes sedimented at 100,000 x *g* (60 min). An acetone powder was prepared by homogenization of the microsomes with acetone at -10°C. This step was repeated and the acetone precipitate extracted with n-butanol at -10°C. Butanol was subsequently removed by acetone and diethylether extractions and the powder dried. Acetone powders were extracted by stirring with 50 mM ammonia buffer (pH 8.5) for 60 min at 0°C, followed by centrifugation and ammoniumsulphate fractionation. A fraction obtained between 40 and 70% saturation was then subjected to molecular sieving on a column (70x1.5 cm) with Sephacryl S-200 (Pharmacia), which was equilibrated and eluted with 50 mM Tris-HCl buffer of pH 8. The monooleoyl-hydrolyzing activity emerged in a sharp peak preceded by a small shoulder. The bulk of this material was found to have a molecular weight of about 60,000, since it was eluted just after bovine serum albumin, as used in a calibration study. Finally, the hydrolase was purified on DEAE Sephadex A-50 (Pharmacia) equilibrated with 50 mM Tris-HCl and eluted stepwise with 1.5 bed volumes of 50 mM, 100 mM and 200 mM Tris-HCl of pH 8. The enzyme activity emerged in the last fraction as a single peak. Polyacrylamide gel electrophoresis (5% gels in rods in 25 mM Tris - 190 mM glycine of pH 8.5) revealed two bands (Rf's 0.4 and 0.5). The main enzyme activity was measured in the first band. The purification is briefly summarized in Table I.

Preparation of antibody

0.3 mg protein of the purified rat liver microsomal enzyme

TABLE I

PURIFICATION OF NON-HEPARIN RELEASABLE GLYCEROL MONOESTER HYDROLASE

Details of the purification steps and enzyme activity measurements are mentioned under METHODS. Enzyme activity is expressed as µmol glycerol released from monooleoylglycerol per min (U).

Fraction	Tot.protein (mg)	Tot.activity (U)	Spec.activity (mU/mg prot.)	Purification factor
Whole homogenate	1429	156	109	1
Microsomes	241	135	559	5
40-70% (w/v) amm. sulphate cut of acetone powder extract	29	53	1821	17
Molecular sieving (Sephacryl S-200) (3 topfractions of 2 ml each)	3.8	18	4729	43
DEAE-Sephadex 0.2 M Tris-HCl eluate (3 topfractions of 2 ml each)	0.7	10	14187	130

was mixed with Freund's adjuvant (1:1) and injected into the foot pads of a rabbit. After 10 days a booster injection was given intra muscularly and this was repeated twice, after which blood was collected by heart puncture.

Enzyme activity measurements

In the monoglyceridase activity measurements reported monooleoyl 2-[^{3}H]-glycerol was used as the substrate[5]. In a few experiments with rat adipose tissue acetone powder extracts and extracts of microsomes from rat liver and rat small intestinal epithelial cells virtually identical activities were measured when monooleoylglycerol was replaced by 2-monopalmitoylglycerol (not shown). Each test contained 2.5 mM monoglyceride, 5 mg bovine serum albumin, 0.1 M Tris-HCl (pH 8.5) and 10-100 µl enzyme sample in a final volume of 0.5 ml. After 10 min at 37°C, the reaction was stopped with 0.5 ml 10% trichloroacetic acid which precipitates the unhydrolyzed long-chain monoglycerides[15]. 0.5 ml supernatant was counted in "Instagel". When lipoprotein lipase or liver postheparin

lipase activities were measured (see footnote and Fig. 2), this was done with tri[1-^{14}C]-oleoylglycerol, emulsified with gum acacia (5% w/v), as the substrate, essentially as described by Ehnholm et al.[16], in the presence of 0.1 or 1.0 M NaCl, to distinguish salt sensitive lipoprotein lipase from salt resistent liver lipase[6,7].

RESULTS AND DISCUSSION

The glycerol monoester hydrolase of rat small intestinal endoplasmic reticulum is not inhibited by the antibody prepared in our laboratory against heparin-releasable liver lipase[17]. This also holds for the non-releasable glycerol monoester hydrolase from rat liver[5]. Hence it is likely that non-releasable monoglyceridase activity is different from the heparin-releasable liver activity, which was the conclusion of our previous paper[5]. Yet it is possible that the protein moieties are related, but that immunological, substrate specificity and kinetic differences[5] are induced by glycosylation, phosphorylation or otherwise modification of the protein. Therefore, we decided to purify non-releasable rat liver microsomal glycerol monoester hydrolase, to raise an antibody against this protein and to extend our immunological studies. The enzyme purification is briefly depicted in Table I. Microsomes were prepared as the starting material, since neutral esterases are enriched in these particles. In fact, neutral esterase activity is a marker for microsomes[18]. An antibody raised against this non-releasable enzyme was found to react neither with heparin-releasable monooleoylglycerol hydrolase activity of a perfusate of a rat liver, obtained by in vitro perfusion of liver with 5 I.U. heparin per ml perfusion medium[3], nor with the activity contained in postheparin serum of an intact rat (Fig. 1). An antibody prepared against postheparin liver lipase[2] gave almost complete inhibition of postheparin liver perfusate and partial inhibition of total postheparin rat plasma (Fig. 1, comp. also refs. 3, 5 and 8). The monooleoylglycerol hydrolase activities (or 2-monopalmitoylglycerol hydrolase activity, which gave similar results; not shown) of acetone powder extracts of microsomal preparations of rat liver, of isolated rat small intestinal villus cells[19] and of whole acetone powders of epididymal fat pads[13], were all found to be inhibited by antiliver microsomal glycerol monoester hydrolase (Fig. 1) and not by antiheparin releasable liver lipase (Fig. 1). Hence, it is likely that the monoacylglycerol hydrolase(s) that occur in the organs tested are structurally more related to each other than to heparin-

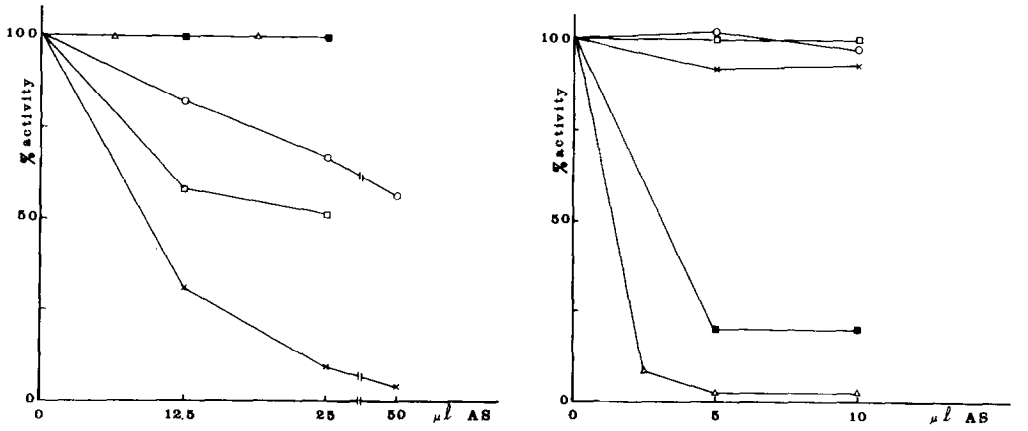

Fig. 1 Immunological relationship between non-releasable monooleoylglycerol hydrolases of different rat tissues. Left: titration with antiserum against purified non-releasable rat liver glycerol monoester hydrolase. Right: titration with antiserum against heparin-releasable liver lipase[2,3]. Equal activities (25 mU) of enzyme samples were preincubated for 3 h at 4°C with the indicated amounts of antiserum (AS). Control serum was used to match the different amounts of antiserum. After centrifugation for 4 min at 15,000 x g, assays were carried out as described under METHODS. Preincubation with control serum did not cause inactivation. Control activities, as indicated, were set 100%. Acetone powder extracts (comp. METHODS) of liver microsomes (1 U/ml) x-x; small intestinal villus cell microsomes[19] (1 U/ml) □-□ or epididymal fat pads[13] (1 U/ml) o-o. Liver perfusate (in vitro perfusion with 200 I.U. heparin in 40 ml Krebs-Ringer bicarbonate buffer, activity 230 mU/ml) Δ-Δ. Postheparin plasma (obtained after in vivo injection of 200 I.U./kg rat; activity 4000 mU/ml) ■-■.

releasable enzymes that are localized in heparin accessible spaces.

The present finding of similarity of glycerol monoester hydrolase(s) in various organs of rat also holds for pig. It can be seen from Fig. 2 that an antibody prepared by Nieuwenhuizen et al.[20] with activity against pig adipose tissue lipases A and B inhibits monooleoylglycerol hydrolase activities of acetone powder extracts of pig liver and small intestinal mucosa microsomes completely. It does not inhibit mono- or trioleoylglycerol hydrolysis in postheparin pig plasma in the absence or presence of 1 M NaCl (Fig. 2). An antibody prepared by Serrero et al. against a highly purified pig intestinal lipase[21] also showed cross reactivity with liver microsomal monoglyceridase activity (not shown).

It is clear from the figures presented that the antibody

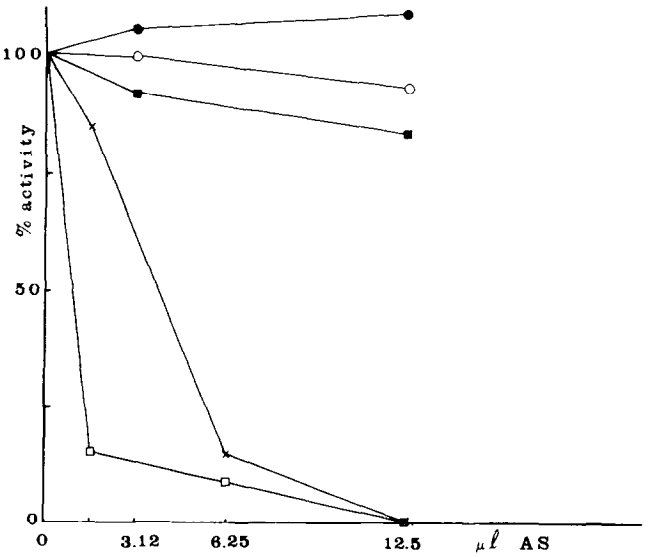

Fig. 2 Immunological relationship between glycerol monoester hydrolases of various pig tissues. The antibody used was prepared by Nieuwenhuizen et al.[20] against purified pig adipose tissue lipase. Activities were measured with monooleoylglycerol substrate (comp. METHODS and Fig. 1), except when lipoprotein lipase was tested. In that case the substrate was trioleoylglycerol (METHODS); 0.1 M NaCl (o-o) or 1 M NaCl (●-●) were present as indicated. Equal activities (10 mU) of enzyme samples were preincubated with the indicated amounts of antiserum (AS) and centrifuged (Fig. 1).

Monoglyceridase activities of liver microsomal acetone powder extract x-x, intestinal microsomal acetone powder extract from a small intestinal scraping (after repeated rinsing of the mucosal surface with cold saline) □-□ and postheparin plasma (collected 6 min after the injection of 100 I.U. heparin per kg pig) ■-■ are shown. 100% of the monoglyceridase activity corresponded to 400 mU/ml enzyme sample and 100% triglyceridase activity to 250 mU/ml enzyme sample.

effectivity varies between the various organs. Therefore it is concluded that the enzymatic activities in the tissue studied are catalyzed by related (iso)enzymes, that differ immunologically from heparin-releasable enzymes completely. There is species specificity since at least the antibody against the pig adipose tissue glycerol monoester hydrolase[20] was not found to inhibit the rat liver enzyme (not shown). The similar lipase-like immunoreactivity of pig adipocytes, myocardium and aorta, as found histochemically by Nieuwenhuizen et al.[20], also suggests the presence of structually related lipases (which according to the experiments presented in Fig. 2

hydrolyze long-chain monoacylglycerol esters) in various organs. It remains to be determined whether the similar non-releasable monoglyceridase activities of the various organs described are due to related isoenzymes or to one enzyme with different availability of antigenic determinants, when isolated from different tissues. Louvard et al.[22] have shown that different amounts of antibodies may be required to precipitate membrane-bound and free aminopeptidase, which is partly due to different availability of antigenic determinants.

Generally, a weak long-chain monoacylglycerol ester hydrolase activity is also found in preheparin serum. In pig, the serum activity is almost negligible and in rat the total activity amounts to about one U, while the long-chain monoglyceridase activity of rat liver amounts to 400 U/10 g liver, 15% of which is heparin-releasable[5]. The heparin-releasable activity may be more favourably localized than the non-releasable activity for the breakdown of plasma 2-monoglycerides that arise during lipoprotein lipase action upon very low density lipoproteins and chylomicrons. The non-releasable enzyme in liver endoplasmic reticulum may supplement plasma lipoprotein breakdown, while the similar enzymes of small intestinal epithelium and adipose tissue mainly have different functions: hydrolyzing monoglycerides from exogenous and endogenous triglyceride sources, which arise during pancreatic and hormone-sensitive lipase actions, respectively. The immunological similarity of these enzymes of liver, small intestine and adipose tissue on the one hand and the immunological difference of these enzymes from the heparin-releasable liver lipase strengthen the opinion[5] that the non-releasable liver enzyme is not a precursor of the heparin-releasable one.

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