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BINDING OF LIVER LIPASE TO PARENCHYMAL AND NON-PARENCHYMAL RAT LIVER CELLS

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SUMMARY The present paper describes the in vitro binding of liver lipase partially purified from postheparin rat serum to isolated liver cells. The binding of liver lipase to non-parenchymal liver cells was 100 times more, per mg cell protein, than to parenchymal cells. The in vitro bound lipase to non-parenchymal cells was largely (80%) releasable by heparin. The presented data suggest that in vivo the heparin-releasable liver lipase is mainly (85-92%) located at the surface of non-parenchymal cells.

INTRODUCTION

Rat liver contains different triacylglycerol hydrolase activities[1,2]. In vitro perfusion of the liver with a heparin-containing medium results in the release of more than 80% of the neutral lipase activity[2]. After heparin administration in vivo a lipase with the characteristics of the liver lipase is found in the plasma[3-5]. The function of this liver lipase in metabolism nor its localization in the liver are known. The releasability of the liver lipase by heparin into the bloodstream suggests an extracellular localization, analogous to lipoprotein lipase in extrahepatic tissues[6]. The liver, however, is not a homogeneous tissue; it consists of parenchymal and non-parenchymal cells (mainly Kupffer and endothelial cells). Earlier studies, initiated to detect the cellular localization of liver lipase were hampered by the finding that during cell isolation the major part of the enzymatic activity is lost[7,8]. Subsequent cultivation or incubation of isolated cells were not able to restore this activity. However, it might be possible that, although the enzyme activity is lost upon cell isolation, the cellular binding properties for liver lipase are not affected. For this reason we purified liver lipase from rat serum and studied the in vitro binding of this enzyme to the different liver cell types.

MATERIALS AND METHODS

Normal fed male Wistar rats (200-250 g) were used. Heparin-releasable liver lipase was purified from rat plasma, collected 3-5 min after intravenous injection of 50 I.U. heparin (Thromboliquine, Organon, Oss, The Netherlands). 8 to 10 ml plasma were applied to a Sepharose-heparin column

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(10 x 1.5 cm). The column was washed with 50 ml 0.2 M NaCl, 50 mM Tris-HCl pH 8.0. The lipase activity was eluted with the same buffer containing 0.8 M NaCl instead. Fractions of 1 ml were collected. The fractions containing the highest lipase activity were combined and passed through Sepharose-G25 (20 x 1.5 cm) and eluted with "incubation buffer" (see later). The fractions containing the highest lipase activity were again combined. These preparations in which the liver lipase is purified about 200-fold are designated as "liver lipase" in the text. Parenchymal cells and a preparation enriched in non-parenchymal liver cells were isolated from rat liver by collagenase treatment and differential centrifugation, exactly as described elsewhere[9]. The non-parenchymal cell preparation was the NPC_2 fraction as described in ref. 9. Protein contents of the non-parenchymal preparations were corrected for parenchymal contamination[9].

Incubations of liver cells with liver lipase were carried out in a buffer medium containing 137 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2H\ PO_4$, 1.5 mM KH_2PO_4, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$ and 6.7 mM glucose (pH 7.4). Cell isolations and incubations were carried out in siliconized (Siliclad, Clay Adams) glassware. During the purification procedure of the liver lipase, palmitoyl-CoA was used as the substrate[10] to measure its activity. The activities in the binding experiments given in the present paper were obtained with glycerol[^{3}H]triolein as the substrate. For the radioactive assay glycerol[^{3}H]triolein ($\sim$ 6 µCi) was sonified in an icebath with 35 mg triolein in 2.5 ml neutral gum acacia solution (0.5% w/v). The emulsion was mixed with 3 ml 10% defatted bovine serum albumin, 0.5 ml 1 M Tris-HCl pH 8.5, 0.125 ml 5 M NaCl and 1.375 ml H_2O. 150 µl of the mixture was incubated with 100 µl sample for 30 min at 30°C. The reaction was stopped and the free fatty acids formed extracted as described by Belfrage and Vaughan[11]. 1 mU lipase activity represents the release of 1 nmole free fatty acid/min from the triolein substrate.

RESULTS

Isolation of parenchymal and non-parenchymal cells from rat liver resulted in a loss of 80 to 90% of the neutral triacylglycerol lipase activity found in the liver before isolation of the cells (TABLE I). Similar losses of neutral liver lipase during cell isolation have been reported by others[7].

Incubation of freshly purified liver lipase for 5 min at 25°C with non-parenchymal or parenchymal cell preparations resulted in binding of lipase activity to the cells (Fig. 1). The amount of lipase activity bound per mg protein was about 20 times higher with the non-parenchymal cell preparation than with the parenchymal cells. The non-parenchymal cells were contaminated with 10 to 15% of parenchymal cells (cell number)[9]. After correction for this contamination it can be seen that non-parenchymal cells bind (per mg protein) approximately 100 times more liver lipase than parenchymal cells (TABLE I). Based on the content of parenchymal (90-95%) and non-parenchymal cells (5-10%) in whole liver[9] it can be calculated that the non-parenchymal cells can bind 85-92% of the total liver lipase (comp. TABLE I). *In vivo* liver lipase is releasable from the liver by heparin. In order to detect whether the *in vitro* binding of liver lipase to non-parenchymal cells reflects the *in vivo* situation, we studied the releasability of the lipase, bound to the non-parenchymal cells

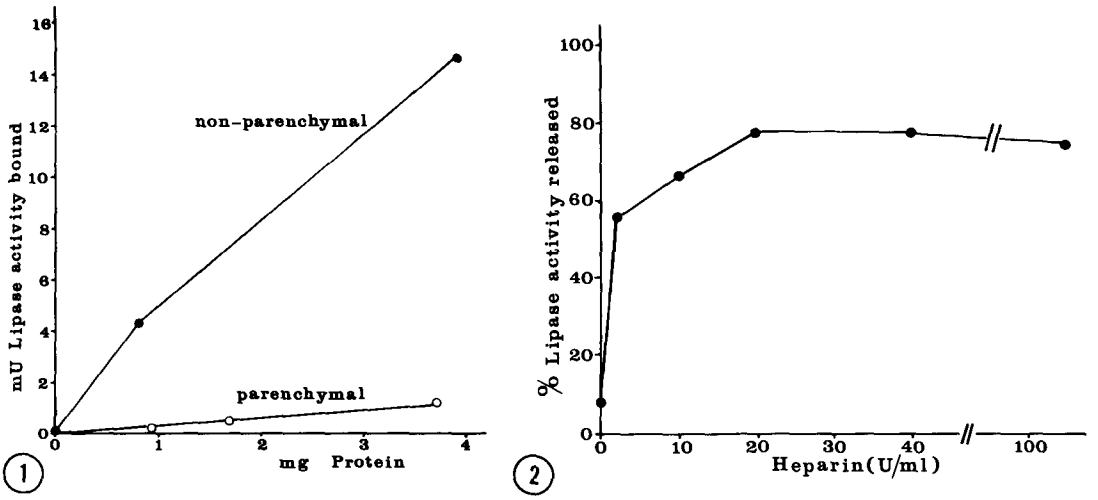

Fig. 1. Binding of liver lipase to parenchymal and non-parenchymal liver cell preparations. Liver cells were isolated as described in ref. 9. Different amounts of cells were incubated in a final volume of 2 ml incubation buffer with 27.2 mU purified liver lipase. After incubation for 5 min at 25°C the cells were separated from the medium by centrifugation (2 min 50 x g_m for the parenchymal cells, 5 min 400 x g_m for the non-parenchymal preparations). The pellets were washed with 2 ml incubation buffer, centrifuged again and then taken up in 0.5 ml buffer. All the samples were sonified for 5 sec at 21 kHz. In the sonified samples lipase activities were measured as described under METHODS. Protein was estimated according to Lowry et al.[13]. The presented data are a typical example of 3 nearly identical experiments (see TABLE I).

Fig. 2. Release of in vitro bound liver lipase by different heparin concentrations. A non-parenchymal liver cell preparation was incubated under standard conditions with liver lipase (comp. Legends to Fig. 1). After washing, the cells were divided in 6 portions each containing 0.6 to 0.7 mg protein. Different concentrations of heparin were added to the samples in a final volume of 1 ml. After incubation for 5 min at 25°C, the media were separated from the cells by centrifugation. Lipase activities were measured in the supernatants and cells after washing. The percentage release was based on the total activities, which are the sum of the released and non-released activities in each sample.

in vitro, by heparin. Fig. 2 indicates that the liver lipase, bound to the non-parenchymal cells in vitro, can be released by 5 min incubation at 25°C with heparin. The release is dose-dependent and results in a release of 80% of the activity bound to the cells.

DISCUSSION

Although the existence of a heparin-releasable lipase activity in the liver of many species is well established, its role, localization and function in lipid metabolism is not clear. The heparin-releasability and the inactivation of the enzymatic activity during cell isolation suggests a predominant extra-

TABLE I

BINDING OF LIPASE ACTIVITY TO PARENCHYMAL AND NON-PARENCHYMAL LIVER CELLS

	Lipase activity (mU/mg) before incubation with liver lipase	Lipase activity (mU/mg) after incubation with liver lipase
Whole liver	1.66 ± 0.20 (n=3)	-
Parenchymal cells	0.21 ± 0.05 (n=3)	0.60 ± 0.07 (n=3)
Non-parenchymal cells	0.30 ± 0.03 (n=3)	41.3 ± 7.8 (n=4)
Binding ratio non-parenchymal cells/parenchymal cells		105
Cellular contribution of lipase binding per total liver (10 g):		
Parenchymal cells (mU)		702-741
Non-parenchymal cells (mU)		4100-8200

Lipase activities of freshly isolated liver cells were determined as described under Materials and Methods and legend to Fig. 1. The values given after incubation with liver lipase are the mean values found in separate experiments. The lipase activity of whole liver was estimated in homogenates of three livers. The homogenates were obtained after perfusion of the liver for 10 min with a Ca^{2+}-free medium. In order to calculate the relative contribution of lipase binding to parenchymal and non-parenchymal cells to the total liver binding, it was assumed that 90-95% of the liver protein is contributed by parenchymal cells and 5-10% by non-parenchymal cells[12]. Values given are means ± S.D.

cellular localization. Such a localization would require binding sites on the surface of cells inside the liver. The presented experiments show that the non-parenchymal cells are able to bind more than 85% of the total lipase activity found in the liver (TABLE I), although these cells only contribute 5-10% of the total liver protein. No further subdivision of the non-parenchymal cell preparation into Kupffer and endothelial cells was made. It may well be that only one of the two different cell types is responsible for this binding leading to an even higher concentration of binding sites per mg of protein. This does not necessarily mean that the enzyme is also synthesized in the non-parenchymal cells. In other experiments (to be published) we found that isolated rat parenchymal cells are able to release lipase activity into the medium so that in the liver an analogous situation may exist as in adipose tissue, where lipoprotein lipase is synthesized in one cell type (the fat cell) and then transported to the functional site at the other cell type (the endothelial cells)[6,14]. That in the presented experiments binding to, rather than uptake of the liver lipase in the potentially endocytotic non-parenchymal cells is studied follows from the following considerations. The association of the lipase activity during incuba-

tion with the cells in vitro is rapid (Fig. 1 shows binding after incubation of the cells with enzyme for 5 min at 25°C, while in unpublished results was found that even after 30 sec 80% of the maximal activity was associated with the cells and the lipase bound in vitro is subsequently largely heparin-releasable (Fig. 2 The availability of preparations of liver cells which contain the physiological amount of liver lipase may be the tool to get further reliable information about the role and function of this enzyme in lipid metabolism. Earlier we concluded[9,12] that the non-parenchymal cells are an important site for liver lipoprotein catabolism. Whether this high uptake of lipoproteins is related to the high binding capacity of non-parenchymal liver cells for liver lipase has t be studied further.

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