SECRETION OF TRIACYLGLYCEROL HYDROLASE ACTIVITY BY ISOLATED PARENCHYMAL RAT LIVER CELLS

H. JANSEN[+,*], C. KALKMAN[+], A. J. ZONNEVELD* and W. C. HÜLSMANN[+]

[+]*Department of Biochemistry I* and **Department of Internal Medicine III and Clinical Endocrinology, Medical Faculty, Erasmus University Rotterdam, PO Box 1738, 3000 DR Rotterdam, The Netherlands*

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1. Introduction

More than 80% of the neutral triacylglycerol hydrolase activity of rat liver can be released from the liver by heparin [1]. Isolation of parenchymal or non-parenchymal liver cells with collagenase results in the loss of > 90% total neutral lipase activity, present in the liver before collagenase perfusion [2,3]. This and the releasability of the enzyme by heparin indicate that the major part of the enzyme is located at the outer surface of the cells present in liver tissue. Such a localization suggests a role of the lipase in extracellular lipid metabolism, e.g., in the hydrolysis of serum lipid, but a definite role of the lipase in lipid metabolism has not been proven yet. A major problem in the study on the function of the lipase is the lack of lipase activity in isolated liver cells. This study was undertaken to see whether the lipase activity could be restored in isolated parenchymal cells. Incubation of isolated parenchymal cells for 4 h at 37°C did not increase the cell-associated lipase activity to a considerable extent. When rat serum was present in the incubation mixture, a time-dependent increase in lipase activity was found in the medium, but not in the cells. The lipase activity in the medium could be inhibited almost completely (> 95%) by an antibody raised against heparin-releasable liver lipase isolated from post-heparin rat serum. Secretion of lipase activity into the medium and the activity in the cells were found to be inhibited when the protein synthesis inhibitor, cycloheximide, was present during incubation of the cells.

2. Methods and materials

Normal fed male Wistar rats were used (260–310 g). Parenchymal liver cells were isolated exactly as in [4], with the exception that the cells were washed 4 times with isolation buffer and once with incubation medium (see later) before use. The intactness of the cells was routinely checked before and after incubation by the trypan blue-exclusion test and by leakage of lactate dehydrogenase from the cells. In the experiments presented, > 90% of the cells excluded trypan blue, while ⩾ 20% of the total lactate dehydrogenase activity was found in the media. The medium in which the incubation experiments were carried out was a Ham F10 medium, supplemented with 10 mM PIPES (piperazine-*N*,*N*′-bis[2-ethane sulfonic acid]), 11.2 mM BES (*N*,*N*-bis[2-hydroxyl ethyl]-2 amino ethane sulfonic acid), 8.9 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid), 2.3 mM $CaCl_2$, 12 mU insulin/ml and rat serum when indicated (pH 7.4). Incubations were performed in 50 ml Erlenmeyer flasks in a 95% O_2/5% CO_2, atmosphere at 37°C. The samples were slowly shaken to keep the cells in suspension. Separation of cells from the incubation medium was achieved by centrifugation at 50 × *g* for 2 min at room temperature; the cells were subsequently taken up in the incubation medium. Before enzyme activities and protein concentrations were estimated, the samples were briefly sonified (5 s at 21 kHz). All glassware used was siliconized with Siliclad (Clay Adams, NY). Lipase activities were measured as the release of free fatty

acid from a glycerol [^{3}H]- or [^{14}C]trioleate emulsion, stabilized with gum arabic, at pH 8.5, exactly as in [3]. Lipase activities are expressed in mU (nmol free fatty acids released/min).

Protein contents and lactate dehydrogenase activities were estimated by standard methods.

The antibody preparation used to identify the lipase activity secreted by the hepatocytes was the γ-globulin fraction (720 μg protein/ml) of an antiserum raised against heparin-releasable liver lipase isolated from post-heparin rat serum. It was shown earlier [1] that this antibody preparation inhibits lipolytic activities that are releasable by heparin from rat liver. The lipolytic activities in rat liver that are not releasable by heparin are not affected by the antibody [1].

Cycloheximide was purchased from Boehringer, Mannheim; collagenase and insulin from Sigma, St Louis, and Ham F10 medium from Flow Labs, Irvine.

3. Results

Incubation of freshly-isolated parenchymal rat liver cells at 37°C in a medium containing 20% rat serum (by volume) resulted in an increase of the total lipase activity of the cell suspension (fig.1). Heating of the serum at 56°C for 1 h, nor overnight dialysis against 0.15 M NaCl, decreased the stimulatory effect (not shown). The increase in activity was linear with the incubation time (4 h measured). By separating the incubation medium from the cells, it was found that the increase in total activity could be accounted for by the appearance of lipase activity in the medium. The cell-associated activity remained low. Removal of the cells from the incubation medium and incubation of the cell-free medium for 1 h at 37°C resulted in an inactivation of the activity in the medium of 55% (broken line, fig.1), indicating that during incubation some inactivation occurs. The amount of activity released by different cell preparations was fairly constant (table 1). Variation of the serum content of the incubation medium (table 1) showed that at lower serum concentrations (10% v/v) less activity was secreted into the medium and/or more inactivation occurred. With no serum present, or at a low serum concentration (2% v/v), very low lipase activities were found in the incubation medium (3 h incubation; table 1). The cell-associated activities were under these conditions comparable to the activities when serum was present in the incubation mixture (table 1). Incubation of the 20% serum-containing medium after 3 h incubation, with an antibody preparation raised against heparin-releasable liver

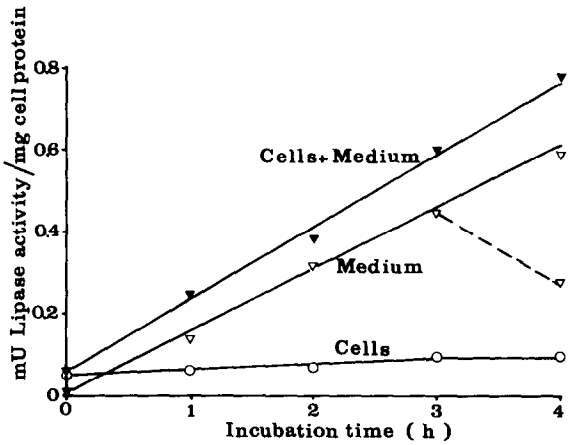

Fig.1. Lipase activities during in vitro incubation of rat liver parenchymal cells. Parenchymal cells were isolated from a normally-fed rat (307 g). The operation started at 9.45 a.m. After isolation, the cells were taken up in ~8 ml incubation medium. Cell suspension (1 ml) was mixed with 9.2 ml incubation medium. From this suspension 0.2 ml was saved for protein determination. To the suspension 2.5 ml rat serum was added. After mixing, 1.5 ml suspension was withdrawn, the rest was incubated at 37°C as in section 2. From the 1.5 ml sample, 0.5 ml was saved for determination of the total lipase activity (i.e., cells + medium) and 1 ml was centrifuged at 50 × *g* for 2 min to separate the cells from the medium. After removal of the cell-free supernatants the cells were taken up in 0.5 ml incubation medium. The same sampling procedure was repeated after 1, 2, 3 and 4 h incubation. From the supernatant obtained after 3 h, 0.5 ml was incubated for 1 h at 37°C in order to study the stability of the lipase activity. All samples were sonified for 5 s before lipase activities were measured in duplicate. The measured lipase activities were expressed per mg cell protein. Total activities, i.e., of cells + medium, are represented by the closed triangles, the medium activities by open triangles and the cell associated activities by the open circles. The broken line represents the activity in the 3 h sample that was incubated without cells. The final concentration of cellular protein in the incubation mixture was 3.1 mg/ml. The figure shown is representative for a number of experiments carried out with different cell preparations (see table 1) under similar conditions.

Table 1
Cell-associated and secreted lipase activities of rat hepatocytes

No. expts	Incubation (min)	Serum in incubation (% v/v)	Lipase activity (mU/mg cell protein) ± SD	
			Cells	Medium
n=4	0	0	0.09±0.03	0.01±0.01
	180	0	0.11±0.04	0.04±0.03
n=2	180	2	0.13 (0.12–0.15)	0.04 (0.04–0.04)
n=2	180	10	0.10 (0.10–0.10)	0.36 (0.33–0.39)
n=5	180	20	0.13±0.06	0.54±0.07
Lipase act. secreted in 3 h (control assay medium)				3.48±5 mU/ml
Lipase act. secreted in 3 h (anti-liver lipase in assay medium)				0.12±7 mU/ml

This table summarizes the results of experiments with different cell preparations. The experiments were carried out as in fig.1 legend except that no samples for 'total' activity were taken and the amounts of rat serum in the incubation media were varied, as indicated. The experiments presented with no serum or serum concentrations < 20% in the medium were paired with one in which 20% rat serum was used, employing the same cell preparation

The lower part shows the total lipase activity in a medium containing 20% rat serum after 3 h incubation with hepatocytes. After centrifugation, 50 μl medium was preincubated for 4 h at 0°C with or without 50 μl antiserum raised in rabbits against purified heparin-releasable rat liver lipase (see section 2), prior to assay of triacylglycerol hydrolyase activity. The mean values of 2 experiments (each carried out in duplicate) are given

lipase of post-heparin rat serum resulted in a nearly complete inhibition (>95%) of the lipase activity of the medium (table 1).

When the protein synthesis inhibitor, cycloheximide, was present during the incubation of the cells in a

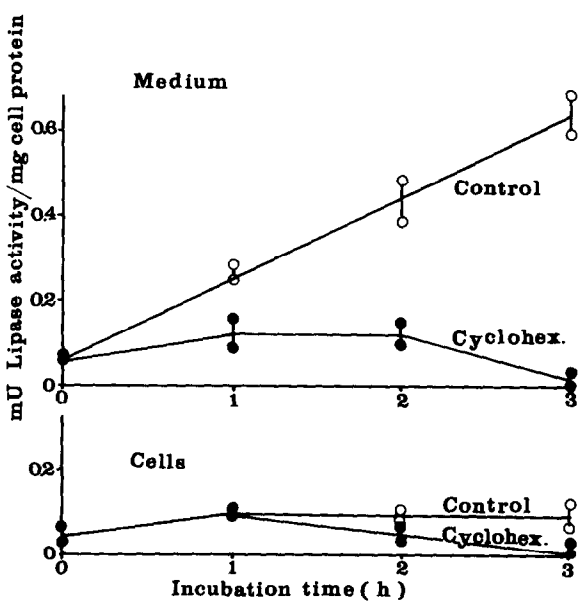

Fig.2. Effect of cycloheximide on the in vitro secretion of lipase activity from parenchymal rat liver cells. Experiments were carried out as in fig.1, with a 20% rat serum-containing medium. Total activities (cells + medium) were not estimated. In the experiments with cycloheximide 10 μg cycloheximide/ml (3×10^{-4} M) was present during the incubations. The incubations were carried out in duplicate. Cell protein was 5.0–5.9 mg/ml incubation mixture. The upper part of the figure shows the activities measured in the media: (○–○) without cycloheximide; (●–●) with cycloheximide present. In the lower part of the figure the cellular activities are given: (○–○) without cycloheximide; (●–●) with cycloheximide. The individual values of the different incubations are given. Zero time values with or without cycloheximide were not different.

serum containing medium, much less lipase activity was measured in the medium (fig.2). The cell-associated lipase activity was equal to the non-cycloheximide treated cells for 1 h, but declined to almost zero in the next 2 h (fig.2).

4. Discussion

Isolated parenchymal rat liver cells were found to secrete triacylglycerol hydrolase activity during incubation in a serum-containing medium. The activity secreted into the medium exceeded the activity in the cells > 4-fold in a 3 h incubation. This indicates that the lipase activity was generated during the incubation rather than just leaked from the cells. The inhibition of the secretion of lipase activity by cycloheximide (fig.2) suggests that some step in the events, leading to the secretion of the enzyme, is dependent on protein synthesis. The secreted enzyme can be newly synthesized, but alternatively some activator of the enzyme or the secretory process itself can be dependent on protein synthesis. From the data of table 1 it can be calculated that ~1380 mU lipase activity were released in vitro from the parenchymal cells of 10 g liver in 4.6 h, assuming that 90% of the liver mass is contributed by the parenchymal cells [4]. In that period (the half-life time of liver lipase [5]) 2655 mU liver lipase activity may be synthesized in vivo, since 10 g whole liver contain 5310 ± 680 mU (n =7), under our assay conditions. Therefore the activity formed by isolated parenchymal cells in vitro (table 1) amounts to ⩾ 50% of that formed in vivo. This percentage may be largely underestimated, as inactivation may occur during the incubations (as discussed above) and also because the conditions used may be suboptimal. The inhibition of the secreted lipase activity by an antibody against heparin-releasable liver lipase indicates that the in vitro secreted enzyme may be identical to the heparin-releasable liver lipase found in post-heparin rat serum. It is of interest that the secreted lipase activity is not bound to the cells. This is in line with our finding that a heparin-releasable liver lipase purified from postheparin plasma does not bind to isolated parenchymal cells but has a high affinity to non-parenchymal liver cells [2]. It may be that in vivo the parenchymal cells synthesize and secrete lipase activity which is then attached to a non-parenchymal, presumably endothelial, cell type in the liver. This situation may be analogous to adipose tissue, where one cell type (the adipocytes) synthesizes and secretes a lipolytic enzyme (lipoprotein lipase) which is then bound to other cells (the vascular endothelium) where the enzyme exerts its metabolic function [6,7]. The secretion of lipoprotein lipase from adipocytes is mediated by at least one serum component, which also holds for the release of lipase from hepatocytes. Whether the same serum factor is operative in the secretion of lipases from adipocytes and hepatocytes, remains to be established. In contrast to the secretion of lipase from adipocytes [8] is that the secretion of lipase from hepatocytes is cycloheximide dependent (fig.2), so that different mechanisms in the assembly and/or secretion of the enzymes from adipose tissue and liver may exist.

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