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Lipoprotein lipase activity in neonatal-rat liver cell types

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The lipoprotein lipase activity in the liver of neonatal (1 day old) rats was about 3 times that in the liver of adult rats. Perfusion of the neonatal liver with collagenase decreased the tissue-associated activity by 77%. When neonatal-rat liver cells were dispersed, hepatocyte-enriched (fraction I) and haemopoietic-cell-enriched (fraction II) populations were obtained. The lipoprotein lipase activity in fraction I was 7 times that in fraction II. On the basis of those activities and the proportion of both cell types in either fraction, it was estimated that hepatocytes contained most, if not all, the lipoprotein lipase activity detected in collagenase-perfused neonatal-rat livers. From those calculations it was also concluded that haemopoietic cells did not contain lipoprotein lipase activity. When the hepatocyte-enriched cell population was incubated at 25 °C for up to 3 h, a slow but progressive release of enzyme activity to the incubation medium was found. However, the total activity (cells + medium) did not significantly change through the incubation period. Cycloheximide produced a time-dependent decrease in the cell-associated activity. Heparin increased the amount of lipoprotein lipase activity released to the medium. Because the cell-associated activity was unchanged, heparin also produced a time-dependent increase in the total activity. In those cells incubated with heparin, cycloheximide did not affect the initial release of lipoprotein lipase activity to the medium, but blocked further release. The cell-associated activity was also decreased by the presence of cycloheximide in those cells. It is concluded that neonatal-rat hepatocytes synthesize active lipoprotein lipase.

INTRODUCTION

Lipoprotein lipase (EC 3.1.1.34) is the enzyme responsible for the hydrolysis of triacylglycerols in plasma chylomicrons and very-low-density lipoproteins (Robinson, 1970). This reaction makes the fatty acids available for use in cellular metabolic processes, and is generally thought to take place in extrahepatic tissues such as heart, skeletal muscle, adipose tissue, lung or lactating mammary gland (Nilsson-Ehle *et al.*, 1980; Cryer, 1981). In recent years, however, many reports have shown the occurrence of lipoprotein lipase activity in the liver of chicken (Bensadoun & Koh, 1977), mouse (Paterniti *et al.*, 1983; Masuno *et al.*, 1984; Olivecrona *et al.*, 1986), cod and rainbow trout (Black *et al.*, 1983). In the rat, lipoprotein lipase activity appears in the liver of newborns, either fed (Chajek *et al.*, 1977; Llobera *et al.*, 1979; Ramírez *et al.*, 1983) or fasted (Grinberg *et al.*, 1985), and in adults, after the administration of cholera toxin (Knobler *et al.*, 1984), after a fat load in fasted animals (Peterson *et al.*, 1985; Vilaró *et al.*, 1986, 1988*a*), after a 24 h fasting period in term-pregnant females (Testar *et al.*, 1985), and after the administration of tumour necrosis factor (Semb *et al.*, 1987).

In extrahepatic tissues the functional fraction of the enzyme is located at the luminal surface of the capillary endothelia (Cryer, 1983), but it is synthesized inside parenchymal cells. Thus, synthesis of lipoprotein lipase has been shown in mesenchymal heart cells (Friedman *et al.*, 1986), preadipocytes (Chajek-Shaul *et al.*, 1985), adult heart myocytes (Severson *et al.*, 1988) and differentiated adipocytes (Vannier *et al.*, 1985; Bensadoun & Marita, 1986; Semb & Olivecrona, 1987). In the chicken liver, which contains lipoprotein lipase activity in the adult, hepatocytes synthesize the enzyme (Jensen *et al.*, 1980). In adult rats, several lines of evidence indicate that, whenever lipoprotein lipase activity appears in the liver, most of the enzyme does not originate in the tissue, but it comes from extrahepatic tissues. Evidence for this is that when labelled lipoprotein lipase is injected intravenously it is taken up by the liver and then degraded quite slowly (Wallinder *et al.*, 1979) being active in the tissue for some time (Wallinder *et al.*, 1984; Vilaró *et al.*, 1988*b*). Also, a good correlation was found between plasma and liver lipoprotein lipase activity in several experimental conditions (Knobler *et al.*, 1984; Peterson *et al.*, 1985; Semb *et al.*, 1987). However, the possibility that the liver synthesizes a small fraction of the lipoprotein lipase that it possesses is not completely excluded. Perfused liver from adult rats incorporates some [^{35}S]methionine into immunoprecipitable lipoprotein lipase (Vilaró *et al.*, 1988*c*), and a small amount of mRNA for lipoprotein lipase was shown in the liver of both mice (Kirschgessner *et al.*, 1987) and guinea pigs (Enerbäck *et al.*, 1988).

In neonatal rats there is no correlation between plasma and liver lipoprotein lipase activity (Vilaró *et al.*, 1988*c*). Neonatal-mouse liver also contains lipoprotein lipase activity, which is decreased in the *cld/cld* mutation (for combined lipase deficiency) (Paterniti *et al.*, 1983; Olivecrona *et al.*, 1986). In the liver of those mutants incorporation of [^{35}S]methionine into immunoprecipitable lipoprotein lipase was found (Olivecrona *et al.*, 1985). The enzyme could hardly come from extrahepatic tissues, since the defect severely impairs release of lipoprotein lipase from cells (Scow & Chernick, 1987).

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Synthesis of the enzyme in perfused neonatal-rat livers has been recently demonstrated by the incorporation of [^{35}S]methionine into immunoprecipitable lipoprotein lipase (Vilaró *et al.*, 1988*c*).

The liver of neonatal mammals contains a large proportion of haemopoietic cells (Emura *et al.*, 1983; Schulze *et al.*, 1984). Because those cell types are absent from the liver during adult life, and, in the rat, the ability of this tissue to synthesize substantial amounts of lipoprotein lipase seems to be a particular feature of newborns, it was possible that those cells were responsible for the synthesis of the enzyme detected in the whole liver (Chajek *et al.*, 1977; Vilaró *et al.*, 1988*c*).

The purpose of the present study was to determine which cell type in the neonatal-rat liver is responsible for the synthesis of lipoprotein lipase.

EXPERIMENTAL

Chemicals

Bovine serum albumin, dithiothreitol, D-glucose, glycerol trioleate, Pipes, Tris, Trypan Blue, glutaraldehyde and paraformaldehyde were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., MEM (minimal essential medium) essential amino acids, MEM non-essential amino acids and BME (basal medium Eagle) vitamin solutions were from GIBCO, Barcelona, Spain. Bovine serum albumin (essentially fatty acid-free), collagenase and Hepes were from Boehringer-Mannheim, Barcelona, Spain. EGTA was purchased from Carlo Erba, Milano, Italy. Insulin was from Novo Industri, Copenhagen, Denmark. Glycerol tri[9,10(n)-^{3}H]oleate was obtained from Amersham International, Amersham, Bucks., U.K. Chicken antiserum to bovine milk lipoprotein lipase, rabbit antiserum to rat liver hepatic lipase and preimmune control sera were generously given by Dr. T. Olivecrona, University of Umea, Sweden. All other reagents were of the highest purity available.

Animals

Rats of the Wistar strain were used. Adult male rats weighed 200–250 g, and were fed *ad libitum* with standard chow diet (65% carbohydrate, 18% protein, 3% fat, 5% fibre, 5% minerals and vitamins, all by wt.) and subjected to a 12 h-light/12 h-dark cycle (starting light at 08:00 h). Newborn rats were used 24 h after birth and were allowed to suckle with their mothers. Experiments were started between 09:00 and 12:00 h.

Hepatocyte isolation and incubation

Adult rats were anaesthetized with sodium pentobarbital (60 mg/kg body wt., intraperitoneally), and hepatocytes were obtained by a modification (Soley & Hollenberg, 1987) of the Berry & Friend (1969) method. Newborn-rat hepatocytes were isolated as follows. After sodium pentobarbital anaesthesia (20 mg/kg body wt., intraperitoneally) the liver was retrogradely perfused (0.8 ml/min, at room temperature) with Ca^{2+}/Mg^{2+}-free Hanks' balanced salt solution containing 0.5 mM-EGTA, buffered at pH 7.4 with 20 mM-Hepes (buffer A), and gassed with O_2/CO_2 (19:1). After 10 min, the perfusion fluid was changed to a $CaCl_2$ (5.0 mM)- and collagenase (0.2 mg/ml)-containing digestion buffer (buffer B: 5.4 mM-KCl, 0.44 mM-KH_2PO_4, 0.98 mM-$MgCl_2$, 0.81 mM-$MgSO_4$, 136.8 mM-NaCl, 1.33 mM-Na_2HPO_4, 5.5 mM-D-glucose, 20 mM-Hepes, pH 7.4 at 37 °C and gassed with O_2/CO_2, 19:1). After 18 min, the liver was carefully excised, cleaned of residual non-hepatic tissues and placed in a plastic flask with 5 ml of $CaCl_2$- and collagenase-containing buffer B. After incubation for 15 min at 37 °C with constant shaking (100 cycles/min), dispersed cells were collected and the residual tissue was rinsed twice with 3.0 ml of buffer C (buffer B supplemented with 1.3 mM-$CaCl_2$ and 10 mg of albumin/ml). The combined cell suspensions were filtered through a double-layered nylon 100-mesh gauze and centrifuged twice for 3 min at 50 ***g***, 3 ml of buffer C being used to suspend cells. The resulting pellet was designated the 'initial fraction'. This fraction was a mixture of hepatocytes and haemopoietic cells. To separate those cell types, the cells were suspended in 3 ml of buffer C and centrifuged twice at 30 ***g*** (1 min) and twice at 15 ***g*** (1 min). The final pelleted cells were designated 'fraction I'. The combined supernatants were centrifuged for 1 min at 40 ***g***, and the resulting supernatant was finally centrifuged for 3 min at 70 ***g***; the collected cells were designated 'fraction II'. In each fraction, cells were suspended in 1 ml of buffer C and hepatocytes and haemopoietic cells were separately counted in a Neubauer haemocytometer. Viable cells were those that excluded 0.2% Trypan Blue. In some experiments, a sample of each fraction (I and II) was immediately pelleted and kept at −20 °C until used for lipoprotein lipase activity measurement. Another sample was washed twice with albumin-free buffer C and used to determine protein (Lowry *et al.*, 1951).

In some experiments, cells of the hepatocyte-enriched fraction (fraction I) were used to study the effect of heparin and/or cycloheximide on the lipoprotein lipase activity of those cells. After isolation, fraction I cells were rinsed twice with buffer D (buffer C supplemented with 2 mM-L-Gln; 0.6 mM-L-Arg; L-Ile, L-Leu, L-Lys, L-Thr and L-Val at 0.4 mM each; L-Cys, L-His, L-Phe and L-Tyr at 0.2 mM each; L-Ala, L-Asn, L-Asp, L-Glu, Gly, L-Met, L-Pro and L-Ser at 0.1 mM each; 0.05 mM-L-Trp; 2 mg of DL-inositol/ml; biotin, D-calcium pantothenate, choline chloride, folic acid, nicotinamide, pyridoxal hydrochloride and thiamin hydrochloride at 1 mg/ml each; riboflavin at 0.1 mg/ml; 10 nM-insulin) and finally suspended at 2.5×10^6 cells/ml with buffer D. Cells (4.5 ml) were incubated in a rotatory water bath at 25 °C, for up to 3 h under O_2/CO_2 (19:1). At zero time cycloheximide and/or heparin solutions were added to give final concentrations of 0.35 mM and 5 units/ml respectively. Control flasks received an identical volume of buffer D. At 0, 45, 90 and 180 min, 1 ml was taken from each flask, the cells were precipitated by centrifugation at 12000 ***g*** for 30 s, and the medium was removed. Both cells and medium were then immediately frozen in liquid N_2 and kept at −20 °C until used to determine lipoprotein lipase activity.

Preparation of cells for scanning electron microscopy

Cells from fractions I and II were fixed in 2.5% glutaraldehyde/2.0% paraformaldehyde in 0.1 M-phosphate (pH 7.5)/0.15 M-NaCl. Before critical-point drying was started, dehydration was carried out in graded series of, first, acetone and then acetone/uranyl acetate. The cells were finally placed on aluminium specimen stubs and sputtered with a 2 nm layer of gold. The samples were examined in a JOEL JSM840 scanning

electron microscope by using an acceleration voltage of 10–30 kV.

Lipoprotein lipase activity assay

To assay lipoprotein lipase activity, livers were homogenized at 4 °C with 10 vol. of buffer E (1 mM-dithiothreitol, 1 mM-EDTA, 0.25 M-sucrose, 10 mM-Hepes, pH 7.4) in a Potter/Elvehjem homogenizer with 20 strokes of a tight-fitting pestle. Isolated cells were suspended with enough buffer E to give 5×10^6 cells/ml and homogenized at 4 °C by sonication (4×30 s at 75 W). Both liver and cell homogenates were centrifuged (10 min, 1000 *g*, 4 °C) and the clear supernatant was used to determine the enzyme activity.

Lipoprotein lipase activity was determined essentially as previously described (Ramírez *et al.*, 1985). The assay mixture contained 0.6 mM-glycerol tri[9,10(n)-^{3}H]oleate (12 Ci/mol), 50 mM-$MgCl_2$, 0.05% albumin (fatty acid-free), 3% (v/v) serum (preheated for 60 min at 50 °C), 25 mM-Pipes, pH 7.5, and 0.02 ml of sample in the final volume of 0.2 ml. Incubation and oleate extraction conditions were as previously described (Vilaró *et al.*, 1988*a*). One unit of enzyme activity was defined as the amount of enzyme that releases 1 μmol of fatty acid/min.

Immunoinhibition experiments

In some experiments, samples (25 μl) of cell homogenates were incubated with an equal volume of either chicken antiserum to bovine milk lipoprotein lipase or rabbit antiserum to rat liver hepatic lipase. The mixture was incubated for 2 h at 4 °C, and then duplicate 20 μl samples were taken to determine lipoprotein lipase activity (see above). As controls, other portions of the sample were incubated in parallel with either preimmune chicken or preimmune rabbit serum.

Calculations

To calculate lipoprotein lipase activity in neonatal hepatocytes (LPL_{Hp}) and in haemopoietic cells (LPL_{Hm}), the following equations were used:

$$LPLI = (HpI \times LPL_{Hp}) + (HmI \times LPL_{Hm})$$

$$LPLII = (HpII \times LPL_{Hp}) + (HmII \times LPL_{Hm})$$

HpI and HpII being the proportion of hepatocytes in fraction I and II respectively, HmI and HmII the proportion of haemopoietic cells in fraction I and II and LPLI and LPLII the lipoprotein lipase activity in either fraction. The same procedure was used to calculate the protein content of both cell types.

RESULTS AND DISCUSSION

Specificity of the lipoprotein lipase activity assay

To assay lipoprotein lipase activity in isolated liver cell extracts, we have used a method (Ramírez *et al.*, 1985) which was developed to determine lipoprotein lipase activity in isolated myocytes from rat ventricles. Most of the methods described to assay lipoprotein lipase activity are not completely specific. When the sample also contains hepatic lipase activity, a variable degree of interference is observed (Peterson *et al.*, 1985; Vilaró *et al.*, 1988*a*). To determine the specificity of the method used, extracts of isolated neonatal-rat liver cells (fraction I; see above) were tested for inhibition by antisera raised against either rat liver hepatic lipase or bovine milk lipoprotein lipase. It was previously shown that these antisera were specific for either enzyme (Vilaró *et al.*, 1988*a*). We found that antiserum to hepatic lipase did not produce any inhibitory effect (Table 1), whereas antiserum to lipoprotein lipase (but not its preimmune control) almost completely inhibited the activity detected in our lipoprotein lipase assay. Those cell extracts contained hepatic lipase activity [107 ± 12 μunits/10^6 cells, assayed by the method of Ehnholm *et al.* (1975)]. So the lack of effect of the antiserum to hepatic lipase on the activity was not due to the absence of hepatic lipase activity in the cell extracts. These results indicate altogether that our assay conditions were specific for lipoprotein lipase activity.

Table 1. Specificity of the lipoprotein lipase assay

Liver cells from 1-day-old rats were dispersed and a hepatocyte-enriched fraction was obtained as indicated in the text. Cells were disrupted by sonication, and 25 μl samples were incubated with an identical volume of either buffer (basal condition) or serum for 2 h at 4 °C, and then used to determine lipoprotein lipase activity. The results are means ± S.E.M. for three different preparations. Statistical comparisons of the specific serum versus its preimmune control were made by the paired Student's *t* test: N.S., not significant; $*P < 0.05$.

Serum added	Lipoprotein lipase activity (μunits/10^6 cells)	*P*
None	71 ± 13	
Rabbit preimmune	81 ± 4	
Rabbit to hepatic lipase	78 ± 14	N.S.
Chicken preimmune	70 ± 23	
Chicken to lipoprotein lipase	2 ± 2	*

Table 2. Lipoprotein lipase activity in liver homogenates

Lipoprotein lipase activity was determined in neonatal (1-day-old)-rat livers which were either unperfused or perfused with a collagenase (0.2 mg/ml)-containing solution for 18 min at 37 °C as indicated in the text. For comparison, the lipoprotein lipase activity of adult livers was also determined. The results are means ± S.E.M. for five animals. Statistical comparisons versus unperfused neonatal-rat liver were made by the unpaired Student's *t* test: $*P < 0.05$; $**P < 0.01$.

Rat livers	Protein (mg/g)	Lipoprotein lipase activity (munits/g)	Lipoprotein lipase activity (munits/mg of protein)
Newborn			
Unperfused	140 ± 6	106 ± 27	0.70 ± 0.19
Collagenase-perfused	123 ± 21	24 ± 4**	0.20 ± 0.04**
Adult	159 ± 10	37 ± 5*	0.24 ± 0.02**

Lipoprotein lipase activity in liver homogenates

As previously described (Ramírez *et al.*, 1983; Peterson *et al.*, 1985), the lipoprotein lipase activity in the liver of neonatal (1 day old) rats was 3 times that in the liver of adult rats when expressed either per g of tissue or per mg of protein (Table 2). It is known that in extrahepatic tissues the functional fraction of the enzyme is located at the luminal side of endothelial cells, from where it can be released by heparin perfusion (Cryer, 1983; Pedersen *et al.*, 1983; Jonasson *et al.*, 1984) or collagenase perfusion (Rajaram *et al.*, 1980). We found that, when livers from normally fed newborns were perfused with collagenase, the tissue-associated lipoprotein lipase activity decreased by 77% (Table 2). We also found that, when newborn-rat livers were perfused with heparin, about 75% of the total activity was released to the perfusion medium (results not shown). The presence of a large fraction of lipoprotein lipase activity at, or close to, the vasculature, as shown by collagenase perfusion and previously by immunofluorescence (Vilaró *et al.*, 1988*c*), strongly supports the hypothesis that the appearance of lipoprotein lipase activity in the neonatal-rat liver might contribute to the accumulation of triacylglycerols observed in the tissue after birth (Ricquier & Hemon, 1976; Jamdar *et al.*, 1978; Ramírez *et al.*, 1983).

Lipoprotein lipase activity in liver cell types

Neonatal-rat liver contains a high proportion of haemopoietic cells (Schulze *et al.*, 1984). In our initial cell suspension they accounted for about 68%, and only 32% of the cells were hepatocytes (results not shown). By sequential centrifugation we resolved two cell populations; the first (fraction I) was enriched in hepatocytes (Table 3), whereas in the second (fraction II) haemopoietic cells were predominant (about 90%). The clear differences, in both size and shape, allow an easy and independent counting of each cell type. Hepatocytes (Figs. 1*a* and 1*b*) were larger (12 μm diameter) and were completely covered by microvilli, which are known to be preserved after isolation of the cells (Ziegler, 1980; Devirgilis *et al.*, 1981). Similar sizes of hepatocytes from fetal rats have been described (Blázquez *et al.*, 1987). Haemopoietic cells (Fig. 1*c*) were a heterogeneous mixture of different cell shapes and were clearly smaller than hepatocytes (4 μm diameter). The heterogeneity of the haemopoietic cells is well documented (Emura *et al.*, 1983).

Expressed per million cells, the lipoprotein lipase activity in fraction I was nearly 7 times that in fraction II (Table 4). This clearly indicates that hepatocytes contain more lipoprotein lipase activity than do haemopoietic cells. The low activity in fraction II could be due either to some very low activity in haemopoietic cells or to the presence of hepatocytes in this fraction (Table 3). Taking together the proportion of each cell type and the lipoprotein lipase activity in either fraction, we sought to calculate the actual activity in both cell types (see the Experimental section). The results indicate that neonatal-rat hepatocytes indeed contain lipoprotein lipase activity (Table 4), whereas the activity in haemopoietic cells was not significantly different from zero. For comparison adult-rat hepatocytes were isolated and the lipoprotein lipase activity was determined. As was found in whole liver, the activity was 2.7-fold higher in neonatal than in adult rat hepatocytes. The difference was higher when the specific activities were compared (10-fold higher in neonatal than in adult hepatocytes; Table 4). In our initial cell suspensions we normally obtain about 40×10^6 hepatocytes per neonatal liver (results not shown). From this and results shown in Tables 2 and 4 we estimated that hepatocytes contained most (about 80%), if not all of the lipoprotein lipase activity found in collagenase-perfused livers.

Table 3. Distribution of hepatocytes and haemopoietic cells in the different cell fractions from neonatal-rat liver

Cell fractions from collagenase-perfused livers were obtained as indicated in the text. In each fraction, the number of cells was determined in a Neubauer haemocytometer, and viable cells were those that excluded 0.2% Trypan Blue. The results are means ± S.E.M. for nine preparations: N.D., not determined (the number of cells was too low to determine viability accurately).

	Fraction I	Fraction II
Hepatocytes		
Total cells ($\times 10^{-6}$)	18.3±2.1	4.4±0.8
Percentage	85.8±1.6	10.6±1.5
Viability (%)	85.6±1.9	N.D.
Haemopoietic cells		
Total cells ($\times 10^{-6}$)	3.0±0.4	36.8±5.3
Percentage	14.2±1.0	89.4±14.6
Viability (%)	N.D.	100

Effect of heparin and cycloheximide

It is known that in adult rats the liver takes up lipoprotein lipase (Wallinder *et al.*, 1979, 1984; Vilaró *et al.*, 1988*b*). Thus it was important to show that the lipoprotein lipase activity detected in isolated neonatal hepatocytes originated there. To do so, we incubated the hepatocyte-enriched fraction (fraction I) for up to 3 h in the presence of a protein-synthesis inhibitor (cycloheximide, 350 μM) and/or heparin (at 5 units/ml), a glycosaminoglycan that promotes the release of newly synthesized lipoprotein lipase to the incubation medium (Cupp *et al.*, 1987; Semb & Olivecrona, 1987). The cells were incubated at 25 °C, since it was shown that at 37 °C, the lipoprotein lipase secreted to the incubation medium rapidly loses catalytic activity (Chohan & Cryer, 1980). We have also found that the lipoprotein lipase activity released to the medium either in the presence or in the absence of heparin was stable at 25 °C for at least 3 h (results not shown). After the 3 h incubation period the viability of the hepatocytes decreased by only about 6%, and there were no differences among incubation conditions (Table 5). At different times, samples were taken to determine lipoprotein lipase activity in the cells and in the medium. The results of this experiment are shown in Fig. 2.

When cells were incubated in an amino acid-, vitamin- and insulin-containing medium, the lipoprotein lipase activity in the cells was maintained during the 3 h of incubation. Cells slowly but continuously secreted lipoprotein lipase activity to the medium (about 0.56 μunits/min per 10^6 cells). It is known that isolated adipocytes from adult rats spontaneously secrete active lipoprotein lipase to the medium (Stewart *et al.*, 1969; Stewart & Schotz, 1974). On the contrary, isolated

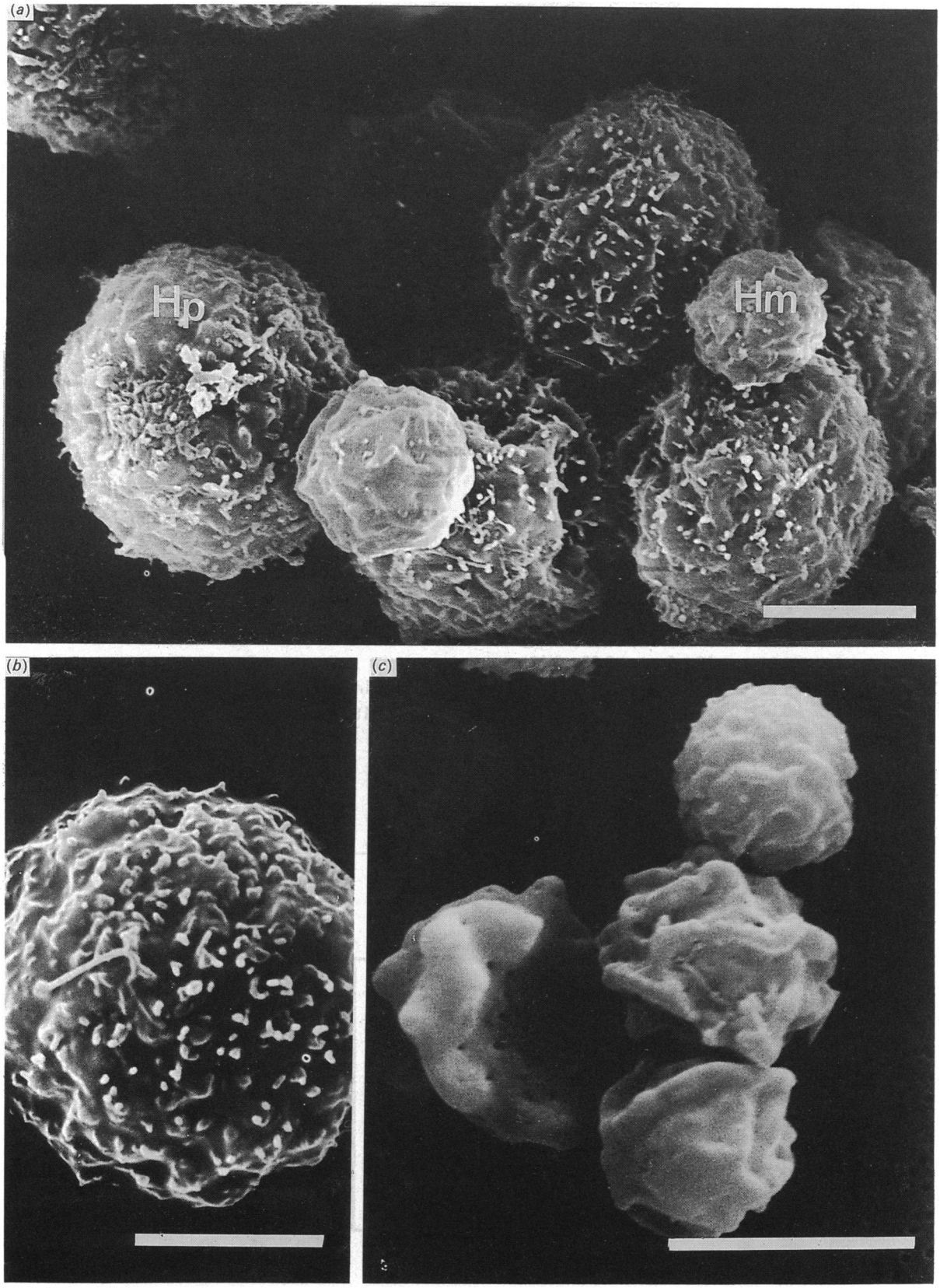

Fig. 1. Scanning electron micrographs of cells from fractions I and II

Cells were processed for scanning electron microscopy as indicated in the text. Panel (*a*) shows cells from fraction I. Note the abundance of microvilli-covered cells corresponding to hepatocytes (Hp) and the presence of some smaller haemopoietic cells (Hm). Panel (*b*) shows a hepatocyte from fraction-I cells. Panel (*c*) shows cells from fraction II. Note the heterogeneity of the morphology of the cells in this fraction. Bar represents 5 μm.

Table 4. Lipoprotein lipase activity in liver cell types

Neonatal (1-day-old)-rat liver cells were dispersed, and hepatocyte-enriched (fraction I) and haemopoietic cell-enriched (fraction II) populations were obtained as indicated in the text. In both fractions the protein content and the lipoprotein lipase activity were determined. To estimate the actual lipoprotein lipase activity in both hepatocytes and haemopoietic cells, those results and the proportion of each cell type in either fraction were used to resolve the equations system shown in the text. The same procedure was used to calculate the actual protein content. For comparison, adult hepatocytes were isolated and the protein content and the lipoprotein lipase activity were determined. Results are means ± S.E.M. for five to six preparations. Statistical comparisons of fraction II versus fraction I were made by the paired t test (**$P < 0.01$; ***$P < 0.001$). Both the protein content and the lipoprotein lipase activity in either hepatocytes or haemopoietic cells were statistically compared with zero (paired t test) (†††$P < 0.001$; ^{NS} not significant). Statistical comparison between neonatal and adult hepatocytes was made by the unpaired Student's t test (‡‡$P < 0.01$; ‡‡‡$P < 0.001$).

	Protein (mg/10^6 cells)	Lipoprotein lipase activity (μunits/10^6 cells)	Lipoprotein lipase activity (munits/mg of protein)
Fraction I	0.28±0.01	80±5	0.27±0.02
Fraction II	0.06±0.01***	12±3***	0.13±0.02**
Hepatocytes	0.32±0.01†††	97±11†††	0.31±0.03†††
Haemopoietic cells	0.05±0.01†††	6±4^{NS}	0.12±0.08^{NS}
Adult-rat hepatocytes	1.29±0.26‡‡	36±4‡‡‡	0.03±0.01‡‡‡

myocytes from rat heart do not secrete lipoprotein lipase activity to the medium in the absence of heparin (Chohan & Cryer, 1980; Severson *et al.*, 1987). The total activity (cells + medium) in the hepatocyte-enriched fraction did not significantly change during the incubation. This maintenance indicates a steady state between enzyme synthesis and degradation, since when the former was blocked by the addition of 350 μM-cycloheximide a continuous decrease in the cell-associated activity was observed, the total activity also being significantly decreased.

Heparin increases the release of lipoprotein lipase

Table 5. Effect of time and incubation conditions on cellular viability

Cells of the hepatocyte-enriched fraction I were incubated for up to 3 h at 25 °C in an amino acid-, vitamin- and insulin-containing medium (see the Experimental section). At zero time a sample was taken for viability measurement, and heparin, cycloheximide or both was added to give final concentrations of 5 units/ml and 0.35 mM respectively. Control flasks received an identical volume of incubation buffer. At the end of the incubation, viability was also tested. Results are means ± S.E.M. for five different experiments.

Incubation condition	Viability (%)
Initial	94±1
180 min	
Control	80±1
Heparin (5 units/ml)	88±4
Cycloheximide (0.35 mM)	87±3
Cycloheximide + heparin	90±1

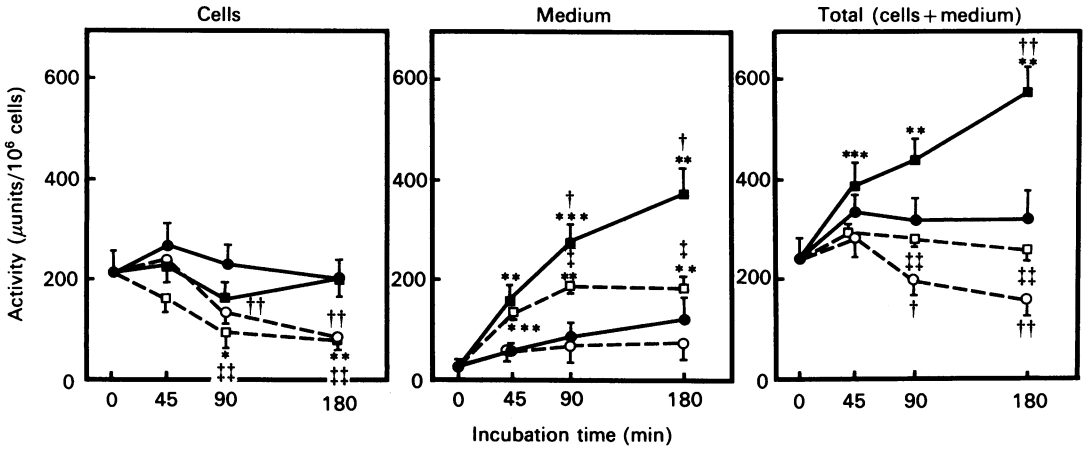

Fig. 2. Effect of heparin and/or cycloheximide on lipoprotein lipase activity

Cells of the hepatocyte-enriched fraction I were incubated in the presence of (●) no addition, (○) cycloheximide, (■) heparin or (□) cycloheximide + heparin as indicated in the text. At different times samples were taken, and the lipoprotein lipase activity in the cells and in the medium was determined. The results are means ± S.E.M. (bars) for five different experiments. Statistical comparisons were made by the paired Student's t test: *P versus zero time; †P versus control group; ‡P versus heparin group. *, †, ‡ $P < 0.05$; **, ††, ‡‡ $P < 0.01$; ***$P < 0.001$.

activity to the medium in a variety of cell types, such as cultured adipocytes (Vannier & Ailhaud, 1986), mesenchymal heart cells (Chajek *et al.*, 1978), peritoneal macrophages (Behr & Kraemer, 1986) or isolated myocyctes from adult rat heart (Chohan & Cryer, 1980; Ramírez & Severson, 1986). In our hepatocyte-enriched fraction, heparin increased the release of lipoprotein lipase activity to the incubation medium (about 2.8 μunits/min per 10^6 cells during the first 90 min). Because the cell-associated activity was unchanged, the total activity progressively increased. As found in other cell types (Chajek *et al.*, 1978; Semb & Olivecrona, 1987; Severson *et al.*, 1988), the heparin-induced secretion of lipoprotein lipase activity to the medium was initially independent of protein synthesis. At 45 min after the simultaneous addition of heparin and cycloheximide, the amount of lipoprotein lipase activity secreted to the incubation medium was not different from the amount released in the presence of heparin alone. However, cycloheximide blocked further release of enzyme activity. Also, the cell-associated activity started to decrease 45 min after the addition of both heparin and cycloheximide. As a consequence, the total activity (cells + medium) did not increase throughout the experiment (contrary to what happens in cells incubated with heparin alone).

Concluding remarks

From the results presented here, we conclude that neonatal hepatocytes synthesize lipoprotein lipase. Evidence for this is: (i) hepatocytes account for most of the activity detected in the liver after perfusion with collagenase, the activity in haemopoietic cells being completely negligible; and (ii) in the hepatocyte-enriched fraction, cycloheximide produces a decrease in both the basal and the heparin-induced total (cells + medium) activity. Because those cells release lipoprotein lipase activity into the incubation medium and heparin stimulates it, we suggest that the vascular pool of the enzyme (the heparin- or collagenase-releasable activity) might originate in the parenchymal cells (hepatocytes), as happens in extrahepatic tissues.

It has been shown that adult-rat hepatocytes do not synthesize active lipoprotein lipase (Leitersdorf *et al.*, 1984). Because neonatal-rat hepatocytes do so, some factors as yet unknown either have to induce neonatal cells to express the enzyme, or have to keep it repressed in adult hepatocytes. Both the factors involved and the mechanism by which they produce the expression of lipoprotein lipase activity in neonatal-rat hepatocytes are important questions raised by the results reported here that deserve further investigation.

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