Different zonal distribution of the asialoglycoprotein receptor, the α_2 -macroglobulin receptor/low-density-lipoprotein receptor-related protein and the lipoprotein-remnant receptor of rat liver parenchymal cells

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Periportal and perivenous parenchymal cells were isolated by the digitonin-pulse perfusion method. The digitonin-pulse perfusion was shown to lead to selective lysis of the correct zone with a straight and sharp border of two to three cells. The mean ratios of alanine aminotransferase activity (a marker for periportal parenchymal cells) and glutamine synthetase activity (a perivenous marker) of periportal to perivenous parenchymal cells were 1.76 and 0.025 respectively. Cells were incubated *in vitro* with 125 I-asialo-orosomucoid (ASOR), 125 I-trypsin-activated α_2 -macroglobulin (α_2 M-T) or 125 I- β -migrating very-low-density lipoprotein (β -VLDL), in order to determine the zonal distribution of the asialoglycoprotein receptor (ASGPr), the α_2 -macroglobulin receptor/low-density-lipoprotein receptor-related protein (α_2 Mr/LRP) and the lipoprotein-remnant receptor, respectively. Maximum binding capacity for 125 I-ASOR on parenchymal cells showed a periportal/perivenous ratio of 0.70. The

periportal/perivenous ratio of $B_{\max}$ values of binding of 125 I- α_2 M-T to parenchymal cells was 1.51. The $B_{\max}$ values of binding of 125 I- β -VLDL, however, were about equal for both cell populations. It is concluded that the maximum binding capacity of the ASGPr on isolated periportal parenchymal cells is 0.70 times that of perivenous parenchymal cells. The 1.51-fold higher expression of the α_2 Mr/LRP on periportal cells, compared with perivenous parenchymal cells, indicates a zonal specialization for the uptake of the suggested multiple ligands. In contrast, the observed homogeneous distribution of the lipoprotein-remnant receptor is in accordance with the suggestion that lipoprotein remnants bind to a specific receptor, which is different from the α_2 Mr/LRP. The zonal heterogeneity in the expression of receptors suggests that receptor-dependent uptake pathways are under zonal control, leading to intrahepatic heterogeneity in the removal of ligands from the blood circulation.

INTRODUCTION

The liver exerts a major role in the removal of ligands from the blood by means of receptor-mediated endocytosis. Over several years evidence has accumulated that parenchymal liver cells consist of sub-populations which show a functional specialization related to their position in the so-called 'metabolic lobulus' (Lamers et al., 1989) (for extensive review see Jungermann and Katz, 1989). These differences are also referred to as metabolic zonation or zonal heterogeneity. Information about the zonal heterogeneity of the expression of receptors on the surface of parenchymal cells is scarce, although indications for the zonal heterogeneity of the asialoglycoprotein receptor (ASGPr) have been obtained. In perivenous (PV) areas a higher activity of dog intestinal alkaline phosphatase (an enzyme with terminal galactose residues) was observed after incubation of liver sections with the phosphatase (Hardonk and Scholtens, 1980). After injection *in vivo* a similar heterogeneity of the phosphatase was evident (Scholtens et al., 1982; Groothuis et al., 1983). Finally, autoradiography of sections of livers perfused *ex vivo* with trace amounts of 125 I-asialo-orosomucoid (125 I-ASOR) also showed a higher PV expression of the ASGPr (Van der Sluijs et al., 1988). In contrast, Evarts et al. (1985) showed that binding of an

antibody to the ASGPr in liver sections was approximately equal in both areas, whereas McFarlane et al. (1990) found that the binding of antibodies to the ASGPr after perfusion *in situ* was higher in periportal (PP) areas. Injection of a small amount of 125 I-ASOR *in vivo* showed that in young rats binding and uptake occurred predominantly in the PP area, whereas in older rats a homogeneous distribution over the PP and PV zones was observed (Daniels et al., 1987).

No information is available on the zonal distribution of the receptor for α_2 -macroglobulin (α_2 M), which is a major plasma glycoprotein with a molecular mass of 718 kDa (Harpel, 1973). α_2 M binds proteinases [e.g. plasmin, chymotrypsin and trypsin; in the last case yielding trypsin-activated α_2 M (α_2 M-T)] in the circulation, which causes a conformational change (reviewed by Travis and Salvesen, 1983; Marshall et al., 1992; Delain et al., 1992). Subsequently, the α_2 M-proteinase complex binds with high affinity (Gliemann et al., 1983) to a specific α_2 M-proteinase receptor (α_2 Mr) on rat liver parenchymal cells (Davidsen et al., 1985), and the ligand is endocytosed and degraded. The α_2 Mr is a 600 kDa protein (Herz et al., 1988) which was shown to be identical with the low-density-lipoprotein receptor-related protein (LRP) (Strickland et al., 1990), and therefore is called the α_2 Mr/LRP. The α_2 Mr/LRP is also suggested to be involved in

Abbreviations used: ASGP, asialoglycoprotein; ASGPr, asialoglycoprotein receptor; ASOR, asialo-orosomucoid; DMEM, Dulbecco's modified Eagle medium; GalNAc, *N*-acetyl-D-galactosamine; KHB, Krebs-Henseleit buffer; LRP, low-density-lipoprotein receptor-related protein; α_2 M, α_2 -macroglobulin; α_2 M-T, trypsin-activated α_2 -macroglobulin; α_2 Mr, receptor for proteinase (trypsin)-activated α_2 -macroglobulin; PP, periportal; PV, perivenous; β -VLDL, β -migrating very-low-density lipoprotein.

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the binding of cholesteryl ester-enriched very-low-density lipoproteins that have β -migrating properties upon agarose-gel electrophoresis, the so-called β -VLDL. β -VLDL is found in the blood of type-III-hyperlipoproteinaemic patients (Frederikson et al., 1969) and in rats fed on a cholesterol-rich diet (Shore et al., 1974; Mahley, 1979). However, additional enrichment of β -VLDL with apolipoprotein E is needed for recognition by the α_2 Mr/LRP (Kowal et al., 1990). β -VLDL competes with chylomicron remnants (Van Dijk et al., 1991) for binding to the putative lipoprotein-remnant receptor. Jones et al. (1984) showed an approximately homogeneous distribution of the lipoprotein-remnant receptor after injection *in vivo* of radioiodinated chylomicron remnants.

Several lines of evidence suggest that the binding sites for α_2 M-T and β -VLDL on total rat liver parenchymal cell populations reside on different molecules, thereby leading to the conclusion that the α_2 Mr/LRP probably differs from the lipoprotein-remnant receptor (Van Dijk et al., 1991, 1992; Ván Berkel et al., 1993). In the present paper we studied the zonal distribution of the ASGPr, the α_2 Mr/LRP and the receptor for lipoprotein remnants on freshly isolated rat liver PP and PV parenchymal cells, isolated by the digitonin-pulse perfusion method as described by Lindros and Penttilä (1985) and Quistorff (1985).

MATERIALS AND METHODS

Materials

BSA (fraction V), collagenase (clostridiopeptidase A, EC 3.4.24.3) (type IV, lot 111H6829), neuraminidase (EC 3.2.1.18) attached to beaded agarose (type VI-A) and *N*-acetyl-D-galactosamine (GalNAc) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). ^{125}I (carrier-free) was purchased from Amersham International (Amersham, Bucks., U.K.). Digitonin/USP (37010; batch 275156 1188) was from Fluka (Perstorp Analytical, Oud-Beijerland, The Netherlands). Dulbecco's modified Eagle medium (DMEM) (powder) was from Gibco (Irvine, Scotland, U.K.). Epon epoxy resin (components as in LX-112 Resin kit) was from Ladd Research Industries (Burlington, VT, U.S.A.). All other chemicals were of analytical grade.

Digitonin treatment and quantification of complexation with cholesterol

A solution of 10 mg of digitonin/ml in Ca^{2+} -free Krebs-Henseleit buffer (Ca^{2+} -free KHB; 24 mM NaHCO_3 , 115.7 mM NaCl , 4.8 mM KCl , 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 , 10 mM Hepes, pH 7.40) was heated for 5 min at 95 °C. The clear solution was left at 4 °C to allow for precipitation of undissolvable digitonin. Precipitated digitonin was removed by centrifugation at 15900 g for 20 min at 4 °C (r_{av} 7.26 cm). The digitonin concentration in the supernatant was determined by complexation with [^3H]-cholesterol as described by Janski and Cornell (1980). ^3H radioactivity was counted in samples of the supernatant in a Packard Tri-Carb 1500 liquid-scintillation analyser. The supernatant was found to contain the cholesterol-complexing equivalent of 8.3 mg of digitonin/ml.

Orosomucoid: isolation, desialylation to ASOR, and radioiodination of ASOR

Isolation of human orosomucoid was performed as described by Whitehead and Sammons (1966). Orosomucoid was desialylated

by incubation with neuraminidase attached to agarose beads, yielding ASOR. Radioiodination of ASOR was performed as described by McFarlane (1958), resulting in a specific radioactivity of 1570 d.p.m./ng of ASOR, of which 99.4 % was found to be 10 %-trichloroacetic acid-precipitable. Protein concentrations of ASOR were determined spectrophotometrically by using $A_{1\text{cm},280}^{1\%}$ 8.9, as described for orosomucoid (Schultze and Heremans, 1966).

α_2 M: isolation, radioiodination and activation with trypsin

Isolation and radioiodination of human α_2 M, and subsequent activation of ^{125}I - α_2 M with trypsin to an ^{125}I - α_2 M-trypsin complex (^{125}I - α_2 M-T) were performed as described previously (Van Dijk et al., 1992). Protein concentrations of α_2 M and (^{125}I - α_2 M-T) were determined spectrophotometrically ($A_{1\text{cm},280}^{1\%}$ 9.1; Gliemann et al., 1983). Specific radioactivity of ^{125}I - α_2 M-T ranged from 430 to 750 d.p.m./ng of protein, of which 98.6 % was found to be 10 %-trichloroacetic acid-precipitable.

β -VLDL: isolation, characterization and radioiodination

Isolation, characterization and radioiodination of rat β -VLDL to a specific radioactivity of 230–290 d.p.m./ng of protein were performed as described previously (De Water et al., 1992). Of total ^{125}I - β -VLDL radioactivity, 93.9 % was found to be 10 %-trichloroacetic acid-precipitable. Protein concentrations of (^{125}I - β -VLDL) were determined by the method of Lowry et al. (1951), with BSA as internal standard.

Visualization of zone-selective cell permeabilization by digitonin

In order to investigate the zonal specificity of the damage inflicted on the respective liver zones by digitonin pulses, visualization experiments were carried out. Rats with a body weight of about 250 g that had fasted for 24 h were anaesthetized by intraperitoneal injection of sodium pentobarbital. ^{125}I -ASOR was injected into the vena cava inferior in a dose of 2 mg/kg body weight (corresponding to 960×10^6 d.p.m./kg) in order to saturate all exposed ASGPr present in the liver. After 15 min a cannula was inserted into the vena porta, through which a 1 min perfusion of the liver with 50 ml of Ca^{2+} -free KHB of room temperature was carried out, followed by a perfusion for 9 min with Ca^{2+} -free KHB (4 °C) at a flow rate of 5 ml/min. A second cannula was inserted into the vena cava superior. Subsequently, a solution containing 9 mg of digitonin and 40 mg of GalNAc in a volume of 2.2 ml was infused for 1 min in the antegrade (or retrograde) direction at a flow rate of 5 ml/min, thus lysing the PP (or PV) areas respectively. GalNAc [an inhibitor of the galactose-specific binding of ^{125}I -ASOR to the ASGPr (Steer and Ashwell, 1980; Lee et al., 1983)] and Ca^{2+} -free KHB [Ca^{2+} is a prerequisite for binding of ^{125}I -ASOR to the ASGPr (Spiess, 1990)] were to prevent re-binding of ^{125}I -ASOR, or fragments thereof, released from the organelles involved in receptor-mediated endocytosis upon lysis by digitonin, to the ASGPr. After 1 min the direction of flow was changed to retrograde (antegrade). The contents of the lysed area were flushed out of the liver for 4 min at 10 ml/min. In order to fix the zonal lysis patterns now obtained, an antegrade perfusion with a fixation buffer, 0.1 M sodium phosphate/ NaCl buffer containing 4 % (w/v) paraformaldehyde, pH 7.40 at 4 °C, was started at a flow rate of 10 ml/min. After 5 min, flow was switched to retrograde, in order to fix the PV areas. Flow through the liver was then stopped, and the liver was excised and put in a glass Petri dish containing ice-cold fixation buffer. Small cubes (approx. 1 mm³)

were cut at random from the liver tissue. The cubes were then rapidly frozen by plunging in solid/liquid Freon 22 so as to avoid formation of ice crystals as much as possible. Frozen cubes were transferred to liquid nitrogen. Further processing (freeze-drying, osmium tetroxide vapour fixation and Epon embedding) was carried out as described under 'Dry' sample preparation in De Haan et al. (1989). By using this method, contact of the tissue with liquids that might extract radioactivity from the tissue is avoided as much as possible. Semi-thin sections ($1\ \mu\text{m}$) were used for light-microscopic autoradiography (Ilford L4 emulsion, Kodak D19b developer). Epipolarization observation of the silver grains was carried out with a Zeiss IM35 microscope, with a PLAN NEOFLUAR 25 lens, offering the opportunity to mix normal and epipolarized light. This enables the simultaneous view of the tissue and the autoradiographic grains, thus making it possible to locate the grains within the liver. Pictures were taken with a Nikkormat camera, using Kodak 400 ASA colour film. Exposure time was 0.5 s.

Control sections were obtained from livers of rats that had received the same dose of ^{125}I -ASOR and were perfused in accordance with exactly the same perfusion scheme, but were not given the digitonin pulses.

Isolation and enzymic characterization of PP and PV parenchymal cells

Male Wistar rats with a body weight of 220–280 g were used. Rats were fed *ad libitum* with standard chow and water.

PP or PV parenchymal cells were isolated by digitonin–collagenase perfusion as described by Lindros and Penttilä (1985) and Quistorff (1985). Instead of infusing digitonin for a certain period of time, a loop containing 18.3 mg of digitonin in a volume of 2.2 ml was flushed at a flow rate of 5 ml/min. After 1 min, the direction of flow was changed and the flow rate was increased to 20 ml/min, in order to wash the cellular contents of the lysed area out of the liver without affecting the parenchymal cells that were to be isolated. The digitonin pulse led to the typical destruction patterns (Quistorff et al., 1985; Lindros and Penttilä, 1985) of the PP or the PV liver areas when the flow was antegrade or retrograde, respectively. Livers showing uneven destruction of either zone as a result of the digitonin pulse were discarded. After 5 min, a recirculating collagenase perfusion (0.04% collagenase and 1 mM CaCl_2 in Ca^{2+} -free KHB) was started at a flow rate of 47 ml/min. After 9 min a 1 min perfusion with Ca^{2+} -free KHB at room temperature was started. Flow through the liver was then stopped, and the liver was excised and placed on ice. Glisson's capsule was gently opened to set free the parenchymal cells. After some minutes ice-cold KHB + 0.3% BSA (containing 2.54 mM CaCl_2) was added. The cell suspension thus obtained was filtered through an 80 μm -pore nylon gauze and subsequently centrifuged for 30 s at 4°C and 50 g (r_{av} , 9.8 cm), and was allowed to come to a halt in 7 min without use of the brake. The pellet was washed six times with KHB + 0.3% BSA and suspended in an equal volume of DMEM + 2% BSA. Of this suspension, 50 μl was added to each incubation. Viability of parenchymal cell preparations was determined by exclusion of Trypan Blue. For enzymic characterization, parenchymal cell suspensions were stored at -80°C . Protein concentrations of parenchymal cells were measured as described by Lowry et al. (1951), with BSA as internal standard.

Cell preparations were assayed for the marker enzymes alanine aminotransferase (L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2) activity as described by Hørder and Rej (1983) and glutamine synthetase [L-glutamate–ammonia ligase (ADP), EC 6.3.1.2] activity as described by Levintow (1954) with the

Figure 1 Lysis of rat liver zones by a digitonin-pulse perfusion

At 15 min after injection *in vivo* of ^{125}I -ASOR, rat liver was perfused with a digitonin pulse, fixed and prepared for autoradiography. (a) Liver section illuminated with normal light. (b) Illumination of the liver section with epipolarized light causes autoradiographic grains to light up as bright dots. Magnification $\times 217$.

following modifications: incubations were carried out in a final volume of 500 μl containing 40 mM L-glutamine, 20 mM hydroxylamine hydrochloride, 0.127 mM ADPNH_2 , 1.6 mM MnCl_2 , 20 mM Na_2HAsO_4 and 40 mM imidazole (final concentrations), pH 6.8 at 37°C . Incubations were stopped by adding 1.0 ml of a solution containing 1.82 M HCl, 87.6 mM HClO_4 and 89.5 mM FeCl_3 .

Cell binding of ^{125}I -ASOR, ^{125}I - $\alpha_2\text{M}$ -T and ^{125}I - β -VLDL *in vitro*

Incubations *in vitro*, and the subsequent handling of PP or PV parenchymal cells after incubations were terminated, were carried

Table 1 Marker enzyme activities of isolated PP and PV parenchymal cells

(a) Parenchymal cell preparations used for incubations *in vitro* with ^{125}I -ASOR. Viability of parenchymal cell preparations was judged by Trypan Blue exclusion, and is given as the percentage of parenchymal cells excluding Trypan Blue. Specific marker enzyme activities of parenchymal cell preparations are given in m-units/mg of protein, where 1 unit corresponds to 1 μmol of substrate formed/min. Levels of significance are indicated, and the PP/PV ratio is given when differences between PP and PV data are significant ($P < 0.05$; P values determined by Student's t test). All results are means $\pm$ S.E.M. for the number of different parenchymal cell preparations shown in parentheses. (b) Parenchymal cell preparations used for incubations *in vitro* with ^{125}I - $\alpha_2\text{M}$ -T and ^{125}I - β -VLDL. The legend is the same as for (a).

	PP	PV	P value	PP/PV ratio
(a)				
Viability (%)	89.8 $\pm$ 1.3 (3)	86.0 $\pm$ 1.4 (3)	0.1248	
Alanine aminotransferase activity	257 $\pm$ 24 (3)	144 $\pm$ 18 (3)	0.0102	1.78
Glutamine synthetase activity	5.6 $\pm$ 2.9 (3)	207 $\pm$ 18 (3)	0.0002	0.027
(b)				
Viability (%)	90.4 $\pm$ 2.8 (5)	90.2 $\pm$ 1.7 (4)	0.9720	
Alanine aminotransferase activity	247 $\pm$ 19 (5)	142 $\pm$ 12 (4)	0.0008	1.74
glutamine synthetase activity	4.5 $\pm$ 1.3 (5)	194 $\pm$ 17 (4)	0.0001	0.023

out as described by Van Dijk et al. (1992). Total binding of ^{125}I -ASOR was determined after 2 h at 4 $^{\circ}\text{C}$, and non-specific binding of ^{125}I -ASOR was determined by depletion of Ca^{2+} from the incubation medium (Spiess, 1990) by addition of 100 mM Mg-EGTA solution to a final concentration of 6.8 mM. The zonal heterogeneity of the ASGPr was studied in one set of experiments. The zonal heterogeneity of the $\alpha_2\text{M}$ /LRP and the lipoprotein-remnant receptor was investigated simultaneously in another set of experiments, to rule out any influence of the isolation procedure itself on the number of receptors. Incubations of cells with ^{125}I - $\alpha_2\text{M}$ -T and ^{125}I - β -VLDL were carried out at 4 $^{\circ}\text{C}$ for 3 and 2 h respectively. During these time-spans binding equilibria were reached (results not shown). Total binding of ^{125}I - $\alpha_2\text{M}$ -T was determined, whereas non-specific binding of ^{125}I - $\alpha_2\text{M}$ -T to parenchymal cells was determined by depleting Ca^{2+} (Van Dijk et al., 1992). Total binding of ^{125}I - β -VLDL was determined and non-specific binding of ^{125}I - β -VLDL was determined by addition of 300 μg of unlabelled β -VLDL/ml. Protein content was determined as described by Lowry et al. (1951) with BSA as internal standard. Radioactivity of samples was determined in a Packard Minaxi γ Auto-Gamma 5000 Series γ -radiation counter. Specific binding was calculated by subtracting the corresponding non-specific binding from each point at which total binding was measured. Specific binding curves were fitted by non-linear regression to a one-binding-site model (GraphPAD; Motulsky, 1987a). K_D and B_{max} values of specific binding, as calculated by GraphPAD, to PP and PV parenchymal cells were tested for the significance of the difference of the means by Student's t test (t-EASE; Motulsky, 1987b).

RESULTS

Visualization of zone-selective cell permeabilization by digitonin

During sample preparation for visualization of ^{125}I -ASOR in liver sections, tissue only came in contact with liquid (possibly leading to extraction of radioactivity) during impregnation with Epon *in vacuo* (De Haan et al., 1989). This leads to the loss of only 0.76 % of total radioactivity from the tissue cubes (results not shown).

Figures 1(a) and 1(b) show a typical liver section (representative of many sections obtained from one liver) after lysis of the PP zone, illuminated with normal light (revealing liver tissue) and epipolarized light (revealing autoradiographic silver grains), respectively. Three areas with their corresponding destruction characteristics can be distinguished. The first area (centre) shows

lysed cells (light appearance, due to the leakage of cell proteins out of the cells), in which very few autoradiographic grains are present. The second area (surrounding the centre) shows lysed cells that still contain autoradiographic grains. This second, transitional, area appears to be only about two to three parenchymal cells wide. The third area (the outer area) shows autoradiographic grains within intact cells. It should be noted that the borders of lysis are quite straight (indicating equal perfusion of the sinusoids with digitonin) and sharp: cells or intracellular organelles are affected by digitonin and have lost their respective protein contents, or are still intact. In the case of selective lysis of the PV zone, the same subdivision in three areas was observed. In control experiments in which no digitonin was infused in the liver, liver zones showed uniform labelling throughout the acinus.

Characteristics of PP and PV parenchymal cells

Cell pellets obtained after the last wash of PP or PV parenchymal cells were of about equal size, but were smaller than observed when isolating total parenchymal cells (results not shown). In the set of incubations *in vitro* with ^{125}I -ASOR, PP and PV parenchymal cell preparations on average were 88 % viable (see Table 1a), as judged by Trypan Blue exclusion. The specific activity of the PP marker enzyme alanine aminotransferase (Shank et al., 1959) was 1.78 times higher in PP parenchymal cells than in PV parenchymal cells, whereas the specific activity of the PV marker enzyme glutamine synthetase (Gebhardt and Mecke, 1983; Häussinger, 1983) in PP parenchymal cell preparations was only 0.027 times that of PV parenchymal cell preparations (Table 1a). In the other set of simultaneous incubations *in vitro* with ^{125}I - $\alpha_2\text{M}$ -T and ^{125}I - β -VLDL, PP and PV parenchymal cell preparations on average were 90 % viable (see Table 1b), as judged by Trypan Blue exclusion. The specific activity of alanine aminotransferase was 1.74 times higher in PP cells than in PV cells, whereas the specific activity of glutamine synthetase in PP cell preparations was only 0.023 times that of PV cell preparations (Table 1b), again indicating good purity of both the PP and the PV parenchymal cell preparations. Absolute values of the parameters mentioned for both sets of cell isolations are very comparable and show consistency of the cell isolation procedure.

Cell binding of ^{125}I -ASOR, ^{125}I - $\alpha_2\text{M}$ -T and ^{125}I - β -VLDL

Binding at 4 $^{\circ}\text{C}$ of ^{125}I -ASOR, ^{125}I - $\alpha_2\text{M}$ -T and ^{125}I - β -VLDL to PP and PV parenchymal cells as a function of concentration are

Figure 2 Binding of ^{125}I -ASOR to isolated PP and PV parenchymal cells as a function of concentration

(a) Isolated PP parenchymal cells were incubated for 2 h at 4 °C with the indicated concentrations of ^{125}I -ASOR. Total binding (Δ) was determined in the presence of 1.8 mM Ca^{2+} . Non-specific binding ($\circ$) was determined in the presence of 1.8 mM Ca^{2+} and 6.8 mM Mg-EGTA. Specific binding ($\blacksquare$) was calculated by subtracting the non-specific binding from the total binding. (b) Isolated PV parenchymal cells were incubated for 2 h at 4 °C with the indicated concentrations of ^{125}I -ASOR. The same procedure was used as for (a). Results shown are means $\pm$ S.E.M. for three different parenchymal cell preparations. When error bars are not visible, errors are within the symbol size.

Figure 3 Binding of ^{125}I - $\alpha_2\text{M-T}$ to isolated PP and PV parenchymal cells as a function of concentration

(a) Isolated PP parenchymal cells were incubated for 3 h at 4 °C with the indicated concentrations of ^{125}I - $\alpha_2\text{M-T}$. Total binding (Δ) was determined in the presence of 1.8 mM Ca^{2+} . Non-specific binding ($\circ$) was determined in the presence of 1.8 mM Ca^{2+} and 6.8 mM Mg-EGTA. Specific binding ($\blacksquare$) was calculated by subtracting the non-specific binding from the total binding. (b) Isolated PV parenchymal cells were incubated for 3 h at 4 °C with the indicated concentrations of ^{125}I - $\alpha_2\text{M-T}$. The same procedure was used as for (a). Results shown are means $\pm$ S.E.M. for five (PP) or four (PV) different parenchymal cell preparations. When error bars are not visible, errors are within the symbol size.

shown in Figures 2, 3 and 4 respectively. Specific binding of all three ligands to PP or PV parenchymal cells was calculated from the total binding by subtracting, at each point at which total binding was studied, the corresponding non-specific binding.

The K_D and B_{max} values obtained from the curves of specific binding of ^{125}I -ASOR, ^{125}I - $\alpha_2\text{M-T}$ and ^{125}I - β -VLDL to PP and PV rat liver parenchymal cells are summarized in Table 2. For all three ligands, K_D values for PP and PV cells were not significantly different. The PP/PV ratio of maximum specific binding capacity of ^{125}I -ASOR to rat liver parenchymal cells was 0.70 (Table 2). The B_{max} value of specific binding of ^{125}I - $\alpha_2\text{M-T}$ to PP cells was 1.51-fold higher than binding to PV cells (Table 2). Thus the zonal gradient of ^{125}I - $\alpha_2\text{M-T}$ binding to rat liver parenchymal cells is the opposite of the zonal gradient observed for the binding of ^{125}I -ASOR. The B_{max} values of binding of ^{125}I - β -VLDL to PP or PV rat liver parenchymal cells were not significantly different (Table 2), thus showing an equal receptor expression in both rat liver parenchymal cell preparations.

Absolute as well as relative values of non-specific binding to PP and PV cells of ^{125}I -ASOR, ^{125}I - $\alpha_2\text{M-T}$ and ^{125}I - β -VLDL were highly comparable (Figures 2, 3 and 4).

DISCUSSION

PP and PV rat liver parenchymal cells were isolated by the digitonin-pulse perfusion technique. Observation of the border of digitonin-induced cell lysis was performed by a 'dry' tissue preparation method, offering excellent retention of radioactivity in the processed tissue. A transition area of only two to three parenchymal cells wide was observed, in which parenchymal cells are damaged but contain intact endocytotic vesicles. Outside this transitional area, parenchymal cells are completely lysed or are intact. This was also observed by Quistorff and Rømer (1989).

The specific activities of alanine aminotransferase (a marker enzyme for PP cells) and glutamine synthetase (a marker enzyme for PV cells) in PP and PV parenchymal cells are in good agreement with values reported by other authors (Lindros and

Figure 4 Binding of ^{125}I - β -VLDL to isolated PP and PV parenchymal cells as a function of concentration

(a) Isolated PP parenchymal cells were incubated for 2 h at 4 °C with the indicated concentrations of ^{125}I - β -VLDL. Total binding (Δ) was determined as described. Non-specific binding ($\circ$) was determined in the presence of 300 μg of unlabelled β -VLDL/ml. Specific binding ($\blacksquare$) was calculated by subtracting the non-specific binding from the total binding. (b) Isolated PV parenchymal cells were incubated for 2 h at 4 °C with the indicated concentrations of ^{125}I - β -VLDL. The same procedure was used as for (a). Results shown are means $\pm$ S.E.M. for five (PP) or four (PV) different parenchymal cell preparations. When error bars are not visible, errors are within the symbol size.

Penttilä, 1985; Quistorff, 1985), thus indicating good enrichment of PP and PV cell preparations.

The K_D values found for ^{125}I -ASOR are in good agreement

with values for ^{125}I -ASOR reported by Weigel and Oka (1983) and for triantennary galactose-terminal oligosaccharides (that are exposed on ASOR after desialylation of orosomucoid) as reported by Lee et al. (1983). We also found that the Ca^{2+} -dependency of the binding of ^{125}I -ASOR is comparable for both cell populations. These similar binding data fulfil the expectation that PP and PV parenchymal cells express the same receptor protein for ^{125}I -ASOR, i.e. the ASGPr. The maximum binding capacity (B_{max}) values that we found in this study, 207 and 293 fmol/mg protein for PP and PV parenchymal cells respectively, indicate that the amount of asialoglycoprotein receptors on the plasma membranes of PP cells is 0.70 times that on PV cells.

Our data on the B_{max} values of the various ligands for both types of parenchymal cells are expressed per mg of cell protein, since preliminary data from our laboratory showed no significant difference between protein content per cell for PP and PV cell preparations (results not shown). Although Väänänen et al. (1983), upon isolation of PP and PV parenchymal cells, observed a difference in diameter for PP and PV parenchymal cells (23 and 25 μm respectively), Gumucio et al. (1986) showed that intrazonal differences in parenchymal cell size are larger than interzonal size differences. These data are supported by the observations by Braakman (1988) and Braakman et al. (1991), who, by fluorescence-activated cell sorting, showed that PP and PV parenchymal cells are of equal size.

Casey et al. (1991) also studied the binding of ^{125}I -ASOR to PP or PV parenchymal cells after pretreating the cells for 45 min at 37 °C in order to increase the number of cell-surface receptors. ^{125}I -ASOR binding was increased 1.68-fold by this preincubation, after which the PP/PV ratio of the B_{max} values was 0.63, a value quite similar to the PP/PV ratio of 0.70 which we observed. Groothuis et al. (1983) also showed preferential PV binding of the asialoglycoprotein dog intestinal alkaline phosphatase *in vivo* and *in vitro*, and Van der Sluijs et al. (1988), by means of perfusions *in situ*, also showed a higher uptake of ^{125}I -ASOR by the PV parenchymal cells.

In general, it can be stated that the digitonin-pulse perfusion technique [as first described by Lindros and Penttilä (1985) and Quistorff (1985)] leads to partial enrichment of the parenchymal cell preparations with either PP or PV parenchymal cells. Results obtained with microdissection techniques followed by microbiochemistry showed a PP/PV ratio of alanine aminotransferase ranging from 1.6 to 5.0 (Jungermann and Katz, 1989), and sampling of periportal and perivenous cellular contents (Quistorff and Grunnet, 1987) even showed a PP/PV ratio of 10. Since the alanine aminotransferase gradient *in vivo* apparently is much steeper than that observed in the PP and PV cell preparations

Table 2 Binding characteristics of the ASGPr, the $\alpha_2\text{M}$ /LRP and the lipoprotein-remnant receptor of isolated PP and PV parenchymal cells

PP and PV parenchymal cells were incubated with ^{125}I -ASOR for 2 h, or with ^{125}I - $\alpha_2\text{M}$ -T for 3 h, or with ^{125}I - β -VLDL for 2 h, at 4 °C. Affinity (K_D) for the receptor and the number (B_{max}) of specific binding sites was determined. Levels of significance (P values) are indicated and the PP/PV ratio is given when differences between PP and PV data are significant ($P < 0.05$; P values as determined by Student's t test). All results are the means $\pm$ S.E.M. for the number of different parenchymal cell preparations shown in parentheses.

		PP	PV	P value	PP/PV ratio
^{125}I -ASOR	K_D (nM)	5.73 ± 0.58 (3)	5.41 ± 0.67 (3)	0.7425	
	B_{max} (fmol/mg of protein)	207 ± 1 (3)	293 ± 29 (3)	0.0192	0.70
^{125}I - $\alpha_2\text{M}$ -T	K_D (nM)	0.87 ± 0.16 (5)	0.88 ± 0.14 (4)	0.9752	
	B_{max} (fmol/mg of protein)	26.6 ± 2.2 (5)	17.6 ± 2.2 (4)	0.0116	1.51
^{125}I - β -VLDL	K_D ($\mu\text{g}/\text{ml}$)	14.3 ± 2.5 (5)	19.1 ± 5.0 (4)	0.3866	
	B_{max} (ng/mg of protein)	192 ± 16 (5)	211 ± 47 (4)	0.6942	

(1.76 in this study), the actual gradient of the ASGPr from the very first PP parenchymal cells to the very last PV parenchymal cells may be even much larger than the difference as described in this paper. It can thus be concluded that PV parenchymal cells express at least a 1.42 times higher amount of the ASGPr on their cell surface than do PP parenchymal cells.

The K_D values for specific binding of ^{125}I - $\alpha_2\text{M}$ -T to PP and PV parenchymal cells are virtually identical. As the non-specific binding for both cell types is almost the same, and binding to both cell types is in accordance with a one-binding-site model, it is very likely that both cell populations express the same receptor for ^{125}I - $\alpha_2\text{M}$ -T. B_{max} values of binding of ^{125}I - $\alpha_2\text{M}$ -T to PP and PV parenchymal cells (26.6 and 17.6 fmol/mg of protein respectively) are in good agreement with values reported previously for total parenchymal cell preparations (Van Dijk et al., 1992) and indicate that the amount of $\alpha_2\text{Mr}$ /LRP on the plasma membranes of PP parenchymal cells is 1.51 times higher than on PV cells.

The K_D values obtained for binding of ^{125}I - β -VLDL to PP or PV parenchymal cells were comparable with the value found by De Water et al. (1992) for total rat liver parenchymal cells. The B_{max} values for binding of ^{125}I - β -VLDL to PP and PV cells also are in good agreement with values reported for total parenchymal cell preparations (Van Dijk et al., 1991, 1992). Our binding results *in vitro*, suggesting homogeneity of the distribution *in vivo* of the lipoprotein-remnant receptor, are in accordance with the results obtained by Jones et al. (1984). They showed a roughly homogeneous distribution in rat liver of association *in vivo* of radiolabeled chylomicron remnants 15 min after intravenous injection. Since β -VLDL and chylomicron remnants compete with each other for the same binding site (Van Dijk et al., 1991), the results of Jones et al. (1984) are in agreement with our conclusion.

Recently it was shown that the cholesterol synthesis *de novo* itself is 1.67 times higher in PP than in PV areas, whereas no zonation of glycerolipid synthesis exists in normal liver (Cheng et al., 1993). Bile-salt uptake from blood and bile acid secretion (Groothuis et al., 1982) and the bile-salt-independent bile flow (Dionne et al., 1990) are also higher in the PP zone. As a result of low PV uptake of bile salts from the blood, the specific activity of cholesterol 7 α -hydroxylase (the rate-limiting enzyme in synthesis of bile acids) and bile acid synthesis are higher in PV parenchymal cells (Ugele et al., 1991). The homogeneous zonal distribution of the lipoprotein-remnant receptor as evident from our data indicates that the delivery of dietary cholesterol by chylomicron remnants to parenchymal cells will reach both PP and PV parenchymal cells evenly, provided that the pathway of uptake coupled to receptor recognition is similarly efficient in PP and PV cells. The different zonal distributions of the ASGPr, the $\alpha_2\text{Mr}$ /LRP and the lipoprotein-remnant receptor suggest that receptor-dependent uptake pathways are under zonal control, leading to intrahepatic heterogeneity in the removal of ligands from the blood circulation.

In relation to the ongoing discussion on the role of the $\alpha_2\text{Mr}$ /LRP as lipoprotein-remnant receptor, we found evidence of a predominant PP expression for the $\alpha_2\text{Mr}$ /LRP, whereas the high-affinity site for β -VLDL recognition (remnant receptor) is found to be distributed homogeneously over rat liver acini. The difference in expression of $\alpha_2\text{M}$ -T and β -VLDL recognition might be utilized as further evidence that ^{125}I - $\alpha_2\text{M}$ -T and ^{125}I - β -VLDL bind to different receptors on the surface of rat liver parenchymal cells.

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REFERENCES

- Braakman, I. (1988) Ph.D. Thesis, University of Groningen, The Netherlands
- Braakman, I., Keij, J., Hardonk, M. J., Meijer, D. K. F. and Groothuis, G. M. M. (1991) *Hepatology* **13**, 73–82
- Casey, C. A., Kragssow, S. L., Sorrell, M. F. and Tuma, D. J. (1991) *Hepatology* **13**, 260–266
- Cheng, H.-C., Yang, C.-M. and Shiao, M.-S. (1993) *Hepatology* **17**, 280–286
- Daniels, C. K., Smith, K. M. and Schmucker, D. L. (1987) *Proc. Soc. Exp. Biol. Med.* **186**, 246–250
- Davidson, O., Christensen, E. I. and Gliemann, J. (1985) *Biochim. Biophys. Acta* **846**, 85–92
- De Haan, F. H. N., Boddé, H. E., de Bruijn, W. C., Ginsel, L. A. and Junginger, H. E. (1989) *Int. J. Pharm.* **56**, 75–86
- Delain, E., Pochon, F., Barry, M. and Van Leuven, F. (1992) *Electron. Microsc. Rev.* **5**, 231–281
- De Water, R., Kamps, J. A. A. M., Van Dijk, M. C. M., Hessels, E. A. M. J., Kuiper, J., Kruijt, J. K. and Van Berkel, Th. J. C. (1992) *Biochem. J.* **282**, 41–48
- Dionne, S., Russo, P., Tuchweber, B., Plaa, G. L. and Yousef, I. M. (1990) *Liver* **10**, 85–93
- Evarts, R. P., Marsden, E. R. and Thorgeirsson, S. S. (1985) *Carcinogenesis* **6**, 1767–1773
- Frederiksen, D. S., Levy, R. I. and Lindgren, F. T. (1969) *J. Clin. Invest.* **47**, 2446–2457
- Gebhardt, R. and Mecke, D. (1983) *EMBO J.* **2**, 567–570
- Gliemann, J., Larsen, T. R. and Sottrup-Jensen, L. (1983) *Biochim. Biophys. Acta* **756**, 230–237
- Groothuis, G. M. M., Hardonk, M. J., Keulemans, K. P. T., Nieuwenhuis, P. and Meijer, D. K. F. (1982) *Am. J. Physiol.* **243**, G455–G462
- Groothuis, G. M. M., Weitering, J. G., Hardonk, M. J. and Meijer, D. K. F. (1983) *Biochem. Pharmacol.* **32**, 2721–2727
- Gumucio, J. J., May, M., Dvorak, C., Chianale, J. and Massey, V. (1986) *Hepatology* **6**, 932–944
- Hardonk, M. J. and Scholtens, H. B. (1980) *Histochemistry* **69**, 289–297
- Harpel, P. C. (1973) *J. Exp. Med.* **138**, 508–521
- Häussinger, D. (1983) *Eur. J. Biochem.* **133**, 269–275
- Herz, J., Hamann, U., Rogne, S., Myklebost, O., Gausepohl, H. and Stanley, K. K. (1988) *EMBO J.* **7**, 4119–4127
- Hørdér, M. and Rej, R. (1983) in *Methods of Enzymatic Analysis*, 3rd edn., vol. 3 (Bergmeyer, H. U., Bergmeyer, J. and Grassl, M., eds.), pp. 444–450, Verlag Chemie, Weinheim
- Janski, A. M. and Cornell, N. W. (1980) *Biochem. J.* **186**, 423–429
- Jones, A. L., Hradek, G. T., Hornick, C., Renaud, G., Windler, E. E. T. and Havel, R. J. (1984) *J. Lipid Res.* **25**, 1151–1158
- Jungermann, K. and Katz, N. (1989) *Physiol. Rev.* **69**, 708–764
- Kowal, R. C., Herz, J., Weisgraber, K. H., Mahley, R. W., Brown, M. S. and Goldstein, J. L. (1990) *J. Biol. Chem.* **265**, 10771–10779
- Lamers, W. H., Moorman, A. F. M. and Charles, R. (1989) *RBC/Cell Biology Reviews* **19**, 5–26
- Lee, Y. C., Townsend, R. R., Hardy, M. R., Lönngren, J., Arnarp, J., Haraldsson, M. and Lönn, H. (1983) *J. Biol. Chem.* **258**, 199–202
- Levitow, L. (1954) *J. Natl. Cancer Inst.* **15**, 347–352
- Lindros, K. O. and Penttilä, K. E. (1985) *Biochem. J.* **228**, 757–760
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mahley, R. W. (1979) *Atheroscler. Rev.* **5**, 1–34
- Marshall, L. B., Figler, N. L. and Gonias, S. L. (1992) *J. Biol. Chem.* **267**, 6347–6352
- McFarlane, A. S. (1958) *Nature (London)* **182**, 53
- McFarlane, B. M., Sipos, J., Gove, C. D., McFarlane, I. G. and Williams, R. (1990) *Hepatology* **11**, 408–415
- Motulsky, H. J. (1987a) GraphPAD Version 2.0, Institute for Scientific Information Software, San Diego
- Motulsky, H. J. (1987b) t-EASE Version 2.0, Institute for Scientific Information Software, San Diego
- Quistorff, B. (1985) *Biochem. J.* **229**, 221–226
- Quistorff, B. and Grunnet, N. (1987) *Biochem. J.* **243**, 87–95
- Quistorff, B. and Rømer, P. (1989) *Histochemistry* **92**, 487–498
- Quistorff, B., Grunnet, N. and Cornell, N. W. (1985) *Biochem. J.* **226**, 289–297
- Scholtens, H. B., Meijer, D. K. F. and Hardonk, M. J. (1982) *Liver* **2**, 14–21
- Schultze, H. E. and Heremans, J. F. (1966) in *Molecular Biology of Human Proteins*, vol. 1, section II (Schultze, H. E. and Heremans, J. F., eds.), p. 188, Elsevier, Amsterdam
- Shank, R. E., Morrison, G., Cheng, C. H., Karl, I. and Schwartz, R. (1959) *J. Histochem. Cytochem.* **7**, 237–239
- Shore, V. G., Shore, B. and Hart, R. G. (1974) *Biochemistry* **13**, 1579–1585
- Spiess, M. (1990) *Biochemistry* **29**, 10009–10018
- Steer, C. J. and Ashwell, G. (1980) *J. Biol. Chem.* **255**, 3008–3013
- Strickland, D. K., Ashcom, J. D., Williams, S., Burgess, W. H., Migliorini, M. and Argraves, W. S. (1990) *J. Biol. Chem.* **265**, 17401–17404
- Travis, J. and Salvesen, G. S. (1983) *Annu. Rev. Biochem.* **52**, 655–709

- Ugele, B., Kempen, H. J. M., Gebhardt, R., Meijer, P., Burger, H.-J. and Princen, H. M. G. (1991) *Biochem. J.* **276**, 73–77
- Väänänen, H., Lindros, K. O. and Salaspuro, M. (1983) *Liver* **3**, 131–139
- Van Berkel, Th.J. C., Bihain, B. E. and Kruijt, J. K. (1993) *Circulation* **88**, I–366, abstr. 1962
- Van der Sluijs, P., Braakman, I., Meijer, D. K. F. and Groothuis, G. M. M. (1988) *Hepatology* **8**, 1521–1529
- Van Dijk, M. C. M., Ziere, G. J., Boers, W., Linthorst, C., Bijsterbosch, M. K. and Van Berkel, Th.J. C. (1991) *Biochem. J.* **279**, 863–870
- Van Dijk, M. C. M., Kruijt, J. K., Boers, W., Linthorst, C. and Van Berkel, Th.J. C. (1992) *J. Biol. Chem.* **267**, 17732–17737
- Weigel, P. H. and Oka, J. A. (1983) *J. Biol. Chem.* **258**, 5095–5102
- Whitehead, P. H. and Sammons, H. G. (1966) *Biochim. Biophys. Acta* **124**, 209–211

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