Location of platelet activating factor binding in rat liver

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Autoradiographs of tissue slices from livers perfused with 1×10^{-9} M-1- $[^3\text{H}]$ octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine ($[^3\text{H}]$ 18:0-*sn*-3-AGEPC) indicate that binding of this agonist is localized in the portal venules in anterograde perfused livers, and in the central venules in retrograde perfused livers. The pattern of silver grains in anterograde perfused liver was not affected significantly by prior exposure to 100-fold excesses of unlabelled 16:0- or 18:0-*sn*-3-AGEPC, 16:0-*sn*-1-AGEPC, or a 1000-fold excess of U.66985. $[^3\text{H}]$ 18:0-*sn*-3-lyso-GEPC produced the same pattern of binding as the acetylated analogue. Measurement of glucose release stimulated by 16:0-*sn*-3-AGEPC demonstrated that the retrograde perfused liver was nearly 1000-fold less sensitive to this compound than the anterograde perfused liver. Exposure of the livers to bovine serum albumin prior to 5×10^{-11} M- $[^3\text{H}]$ 18:0-*sn*-3-AGEPC resulted in inhibition of stimulated glucose release, and decreased both the amount of label retained in the livers and the amount of silver grains over the portal sinusoidal cells without affecting the amount of grains seen over all other regions of the liver. Glucose release from primary monolayer cultures of hepatocytes or suspensions of liver slices was not stimulated by 16:0-*sn*-3-AGEPC. The results suggest that specific binding of $[^3\text{H}]$ 18:0-*sn*-3-AGEPC is restricted to the portal side of the liver microvasculature, the majority of binding is nonspecific, and the biological response to AGEPC requires an intact and perfused vasculature.

INTRODUCTION

Platelet activating factor (1-*O*-alkyl-2-acetyl-glycero-3-phosphocholine, AGEPC) stimulates transient increases of glucose release, glycogen phosphorylase activation and portal pressure, and a transient decrease in hepatic oxygen consumption in the perfused rat liver (Shukla *et al.*, 1983; Mendlovic *et al.*, 1984; Buxton *et al.*, 1984, 1986a). These responses are dose-dependent with a $K_{0.5}$ of 2×10^{-10} M, require extracellular Ca^{2+} , show attenuation during successive exposure to the agonist and occur in the absence of appreciable changes in cyclic AMP, or ADP levels (Buxton *et al.*, 1984, 1986a). The hepatic responses to AGEPC are inhibited by the *sn*-3 modified analogue U.66985 (Buxton *et al.*, 1986b) but not by the *sn*-1 stereoisomer which is biologically inactive (Shukla *et al.*, 1983). In contrast, AGEPC does not increase significantly glucose release or phosphorylase activation (Fisher *et al.*, 1984; Mendlovic *et al.*, 1984) in suspensions of isolated hepatocytes. Further, in response to exposure to AGEPC, hepatocyte suspensions exhibit transient changes in phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 4-phosphate (Shukla *et al.*, 1983; Fisher *et al.*, 1984) in the absence of significant changes of inositol 1,4,5-trisphosphate levels (Charest *et al.*, 1985). These results have prompted the suggestion that the primary site of action of AGEPC in the liver is not at the hepatocyte.

The basic structural units of the liver include the portal vein and subsequent branches (portal venules), the sinusoids, central venules and veins, and lastly the hepatic vein (for review see Campra & Reynolds, 1982). The sinusoids are immediately bounded by a layer of Kupffer and endothelial cells together comprising the reticuloendothelial system. These cells actively clear, by pino- and phagocytotic mechanisms, both biological (e.g. bacterial endotoxin and immune aggregates) and non-biological (e.g. carbon and latex particles) (Widmann *et al.*, 1972; Wisse, 1977; Fahimi, 1982; Praaning-Van Dalen & Knook, 1982) substances. This layer of cells separates the sinusoidal space from the hepatocytes. The walls of the venous system are also made up of cells of the reticuloendothelial system, and evidence from denatured albumin uptake experiments suggest that functional differences exist between periportal and perivenous endothelium (Yokota, 1985). In addition, there is some evidence that Kupffer cells are located at a density gradient highest in sinusoids around the portal tract and lowest around the central veins (see Jones & Summerfield, 1982). The suggestion has also been put forth that blood flow through the various sinusoids may be controlled by endothelial cells located at the junctions of portal or central venules and the sinusoids (McCuskey, 1966). This report also showed that these cells have the potential to constrict or swell in response to some vasoactive substances. Potentially, AGEPC could bind at any or all of these sites. Identification of the primary

Abbreviations used: the most specific form of naturally produced platelet activating factor has the structural formula of 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine. A simplified form of this name is *sn*-3-AGEPC, and this is used throughout the text with a notation as to the chain length of the alkyl residue. Other abbreviations used are: *sn*-1-AGEPC, 3-*O*-alkyl-2-acetyl-*sn*-glycero-1-phosphocholine; *sn*-3-lyso-GEPC, 1-*O*-alkyl-*sn*-glycero-3-phosphocholine; U.66985, 1-*O*-octadecyl-2-acetyl-*sn*-glycero-3-phosphoric acid 6'-trimethylammonium hexyl ester.

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site of specific binding would create a clearer understanding of the mechanism of action of AGEPC in modifying hepatic haemodynamics and metabolism.

This study was initiated in an attempt to identify the location of AGEPC binding in the liver with the hope of clarifying the dichotomy of responses observed in the perfused liver and in isolated hepatocyte suspensions. We report here that the major portion of AGEPC binding in the liver is non-specific and is localized to the liver vasculature. In tandem with glucose release experiments performed using anterograde and retrograde perfused livers, and in suborganismic liver fractions, our results suggest further that the portal vasculature is a strong candidate for the site of specific binding of AGEPC.

EXPERIMENTAL

Materials

sn-3-AGEPC (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) and *sn*-1-AGEPC (3-*O*-hexadecyl-2-acetyl-*sn*-glycero-1-phosphocholine) were purchased from Bachem, Bubendorf, Switzerland. [³H]18:0-*sn*-3-AGEPC (1-*O*-[10,11-³H]octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (67 Ci/mmol) was prepared by tritiation (New England Nuclear) of 1-*O*-(10,11-*cis*)-octadecenyl-2-acetyl-*sn*-glycero-3-phosphocholine (gift from Dr. D. E. Ayer, Lipids Research, The Upjohn Company, Kalamazoo, MI, U.S.A.). The corresponding deacylated derivative, [³H]18:0-*sn*-3-lyso-GEPC, was prepared by base-catalysed methanolysis of the parent compound. U.66985 (1-*O*-octadecyl-2-acetyl-*sn*-glycero-3-phosphoric acid 6'-trimethylammonium hexyl ester) was generously provided by Dr. D. E. Ayer. Latex microspheres (0.6 μ m diam, 2.5% w/v) were from Polysciences Inc., phenylephrine was from Sigma, and bovine serum albumin (fatty acid free) was from Boehringer. All other materials were the highest quality available commercially.

Methods

Livers from male Sprague-Dawley rats (Houston facility of Harlan Sprague-Dawley, 150–200 g body weight fed *ad libitum*, and maintained on a 07:00–21:00 light cycle for 5–10 days) were perfused *in situ* with a non-recirculating perfusion system as described previously (Scholz *et al.*, 1973). The perfusate was Krebs-Henseleit bicarbonate-buffered saline (Krebs & Henseleit, 1932) containing 1.25 mM-CaCl₂, saturated with a gas mixture of O₂/CO₂ (19:1) and maintained at 37 °C. Livers were perfused for 30 min prior to any experimental manipulation. At that time, in some experiments, the direction of perfusion was changed from anterograde (portal to hepatic vein) to retrograde (hepatic to portal vein).

AGEPC binding was assessed as follows. Livers were perfused for 40 min with Krebs-Henseleit buffer alone, then for the following 2 min (40–42 min of perfusion) with [³H]18:0-*sn*-3-AGEPC (45–53 Ci/mmol, 1×10^{-9} M perfusate concentration). The perfusion was continued for a further 0.5, 1.0 and 5.0 min. At each time point a lobe was ligated and treated as previously described (Ison & Sheridan, 1981). Briefly, a section (about 0.5 cm³) of the lobe was removed, mounted on brass tissue holders and immersed in liquid propane. Serial 4 μ m-thick sections (three to five) were retained from every 150–200 slices cut (–35 °C, Harris Wide-Range cryostat)

and placed on glass slides coated with Kodak NTB-2 nuclear tract emulsion. For each experimental condition the resulting autoradiographs were identical, and representative slides are illustrated. Preliminary experiments showed that the tissue could not be prepared for electron microscopic observation, as exposure to fixing and dehydrating agents resulted in complete removal of tissue-associated silver grains due to the lipophilic nature of AGEPC. After exposure for at least 3 weeks the slides were developed, stained with Toluidine Blue (0.1% in water) and photographed at $\times 50$ or $\times 250$ magnification. In some experiments the livers were perfused with 16:0-*sn*-1-AGEPC (1×10^{-7} M), U.66985 (1×10^{-6} M) or unlabelled 16:0-*sn*-3-AGEPC (1×10^{-7} M) after 35 min of perfusion. In others, unlabelled 16:0-*sn*-3-AGEPC (1×10^{-9} M) was infused over the intervals 35–37 and 45–47 min, followed by the infusion of labelled 18:0-*sn*-3-AGEPC at 55–57 min and tissue sampling as usual. In yet other experiments, [³H]16:0-*sn*-3-lyso-GEPC (17.5 Ci/mmol, 1×10^{-9} M) was infused instead of labelled AGEPC.

AGEPC conversion to its lyso-GEPC and alkylacyl-GPC metabolites was assayed in chloroform extracts of duplicate livers perfused, at 25 °C, with 2×10^{-10} M-³H]18:0-*sn*-3-AGEPC for 2 min followed by 8 or 28 min perfusion in the absence of AGEPC. Livers were freeze-clamped prior to extraction, and labelled lipids were analysed as described previously (Miwa *et al.*, 1987) and expressed as fractions of the total label extracted.

Primary monolayers of hepatocytes and suspensions of liver slices were prepared as described previously, with the addition of 0.1% bovine serum albumin in the final suspension medium (Hill *et al.*, 1987). Glucose release from these preparations and perfused livers, in the absence or presence of 16:0-*sn*-3-AGEPC (1×10^{-9} or 1×10^{-7} M) or phenylephrine (1×10^{-5} M), was measured using the method of Bergmeyer *et al.* (1974). Glucose release is expressed as μ mol/h per g wet weight or as nmol/h per 60 mm culture dish. For perfusion experiments the amount of glucose released by agonists was expressed as the maximum observed change in rate above the basal rate of glucose release. All experiments were repeated at least twice. Means and S.E.M. are shown for the glucose release experiments.

RESULTS

Autoradiographs of tissue slices taken from livers exposed to [³H]AGEPC and then unlabelled perfusate for 0.5 min show that the highest density of silver grains is localized to the portal venule vasculature (Fig. 1*a*). The density of grains in the parenchyma, arterioles, bile ducts and central veins is not different from the background density (Fig. 1*a*). The topographic distribution of silver grains was similar for livers perfused for 1.0 or 5.0 min after the 2 min exposure to [³H]-AGEPC (results not shown). The results indicate that the majority of AGEPC infused into the liver binds in the portal vasculature and does not diffuse into the sinusoids over the time course of the perfusion.

Pretreatment of the perfused livers with substances or conditions known to modulate the glucose release induced by AGEPC prior to [³H]AGEPC infusion were ineffective in changing the pattern of grain distribution in liver slices (results not shown). Thus exposure to a 100-fold excess of 16:0- or 18:0-*sn*-3-AGEPC did not inhibit

Fig. 1. Autoradiographs of $[^3\text{H}]sn\text{-}3\text{-AGEPC}$ binding in liver perfused in the anterograde direction

Livers were exposed to $16:0\text{-sn-}1\text{-AGEPC}$ ($1 \times 10^{-7} \text{ M}$) from 35 min and $[^3\text{H}]18:0\text{-sn-}3\text{-AGEPC}$ between 40 and 42 min of perfusion. Tissue was removed at 42.5 min and slices were prepared for autoradiography as described in the text. Photography of the light micrographs show (a) portal triad and (b) central venule; bar = $10 \mu\text{m}$. P, portal venule; C, centrilobular venule.

binding of $[^3\text{H}]sn\text{-}3\text{-AGEPC}$, suggesting that the majority of binding is non-specific. This conclusion is corroborated by the observations that neither an excess of $sn\text{-}1\text{-AGEPC}$ [which does not modulate the increased glucose release stimulated by $sn\text{-}3\text{-AGEPC}$ (results not shown)], nor pre-exposure to two successive infusions of unlabelled $sn\text{-}3\text{-AGEPC}$ prior to $[^3\text{H}]AGEPC$ infusion [a condition which results in successive attenuation of the transient increase in glucose release (Buxton *et al.*, 1984)], nor pre-exposure to U.66985 [at 1000-fold excess, which completely blocks AGEPC-stimulated glucose release (Buxton *et al.*, 1986b)], affect the distribution of grains illustrated in Fig. 1. Further, in experiments where $[^3\text{H}]sn\text{-}3\text{-lyso-GEPC}$ [which is ineffective in stimulating glucose release (Shukla *et al.*, 1983)] was infused instead of $[^3\text{H}]AGEPC$ the distribution of grains was not qualitatively different from that shown in Fig. 1.

Yamashita *et al.* (1983) have shown that total binding of AGEPC to both biological membranes or liposomes can be decreased in the presence of bovine serum albumin. Table 1 shows results from experiments in which livers were perfused with $[^3\text{H}]sn\text{-}3\text{-AGEPC}$ ($5 \times 10^{-11} \text{ M}$) in the absence or presence of 0.25 or 1.25 %

Table 1. Effect of bovine serum albumin on $[^3\text{H}]sn\text{-}3\text{-AGEPC}$ binding and glucose release in the perfused liver

Livers were perfused at 25°C in the absence or presence of bovine serum albumin from 20 min of perfusion and $[^3\text{H}]18:0\text{-sn-}3\text{-AGEPC}$ ($5 \times 10^{-11} \text{ M}$) between 40 and 42 min of perfusion. Tissue was removed at 42.5 min and slices were prepared for autoradiography as described in the text. Perfusion was continued for a total of 60 min. Perfusate samples were collected from 35 to 60 min and analysed for glucose and ^3H as described in the text. Data are expressed as the mean $\pm$ S.E.M. of three perfusions for each albumin concentration. ^3H bound (%) is: [(total d.p.m. infused - total d.p.m. in effluent perfusate)/total d.p.m. infused] $\times 100$, between 40 and 42 min; ^3H washed out (%) is: (total d.p.m. in effluent perfusate/total d.p.m. infused) $\times 100$, between 40 and 60 min; N.D., not detectable.

Bovine serum albumin (g/100 ml)	^3H bound (%)	^3H washed out (%)	Glucose release ($\mu\text{mol/h per g}$)
0	81.0 (2.5)	4.9 (0.8)	35 (15)
0.25	43.5 (3.4)	61.7 (8.3)	15 (5)
1.25	30.6 (1.3)	98.8 (17.5)	N.D.

bovine serum albumin. A low concentration of $[^3\text{H}]sn\text{-}3\text{-AGEPC}$ was used since, with a higher concentration ($1 \times 10^{-9} \text{ M}$), even 1.25 % albumin was unable to perturb either the uptake of ^3H or the glucose release response effected by AGEPC (results not shown). Perfusions were conducted at 25°C to decrease the rate of metabolism of AGEPC. Perfusion at 25°C for 8 min following exposure to $[^3\text{H}]AGEPC$ resulted in 72.4 % (S.E.M. = 10.3 %, $n = 2$), 13.8 % (2.7) and 8.0 % (1.1) of the total extracted label in AGEPC, lyso-GEPC and alkylacyl-GPC respectively. After a total of 28 min perfusion 31.3 % (1.7), 33.3 (5.3) and 19.1 % (2.7) of the total label was found in the same materials. In addition the low concentration of $[^3\text{H}]sn\text{-}3\text{-AGEPC}$ used made it necessary to store the tissue slices on photographic emulsion for 16 weeks prior to development of the autoradiographs. The results show that exposure of the livers to increasing concentrations of bovine serum albumin resulted in decreased uptake of ^3H during label infusion, increased washout of ^3H and decreased the glucose release stimulated by AGEPC. With 1.25 % albumin no stimulation of glucose release was detected, although about 30 % of the infused label was taken up by the livers. Examination of the autoradiographs of tissue slices obtained from biopsies of the same livers taken 0.5 min after perfusion in the absence of label showed that silver grains were present in the portal sinusoids in the absence of albumin (Fig. 2a) but not when 1.25 % albumin was present (Fig. 2b). With 0.25 % albumin qualitatively similar results were obtained as those shown in Fig. 2(a) (not shown). These results suggest that specific binding sites, which result in the glycogenolytic response, are associated with the portal sinusoids.

In addition to the previous attempts to distinguish between specific and non-specific binding of AGEPC in the liver, $[^3\text{H}]sn\text{-}3\text{-AGEPC}$ was infused in the retrograde perfused liver. The autoradiographs illustrated in Fig. 3

Fig. 2. Autoradiographs of [^3H]*sn*-3-AGEPC binding in liver in the absence or presence of bovine serum albumin

Livers were perfused in the absence (a) or presence (b) of 1.25% (w/v) bovine serum albumin from 20 min of perfusion and from 40–42 min with 5×10^{-11} M- $[\text{^3H}]18:0$ -*sn*-3-AGEPC. Tissues were removed at 42.5 min and treated as described in the legend to Fig. 1. The portal triad, with dominant portal venule, and surrounding parenchyma are illustrated; bar = 25 μm .

show that the distribution of silver grains is the opposite of that seen in the anterograde perfusions. The highest density of grains is in the central venules (Fig. 3a). There is some diffusion of grains into the surrounding parenchyma, indicating a higher degree of mobility of AGEPC in the retrograde compared with the anterograde perfused liver. The portal vasculature, arterioles and bile ducts contain a density of silver grains equivalent to background (Fig. 3b).

In attempts to define more clearly the nature of AGEPC binding in the liver, and to determine whether a polarity of the hepatic response to AGEPC exists, the effect of AGEPC on glucose release was measured in anterograde and retrograde perfusions. Fig. 4 shows that infusion of *sn*-3-AGEPC (1×10^{-9} M) during retrograde perfusion does not stimulate a transient increase of glucose output. After a switch back to anterograde perfusion, AGEPC infusion stimulates a transient increase in glucose output, the peak of which is 115 $\mu\text{mol/g}$ per h above the basal glucose release. A transient

Fig. 3. Autoradiographs of [^3H]*sn*-3-AGEPC binding in liver perfused in the retrograde direction

Livers were perfused in the retrograde direction and were otherwise treated as described in the legend to Fig. 1. Illustrated are (b) portal triad and (a) central venule; bar = 25 μm . For definitions of symbols see legend to Fig. 1.

increase of glucose release was always seen immediately following the initial switch from antero- to retrograde perfusion. This likely resulted from a transient decrease in O_2 supply to the liver, since the effluent flow rate decreased by at least 50% following the switch. In some instances a transient visible swelling of the livers paralleled the decrease in flow rate. Within 3–5 s complete recovery of the flow rate was attained. Observation of perfusion pressure also showed transient increases which recovered within 30 s to that observed prior to switching from antero- to retrograde perfusion (not shown). The switch response was variable in magnitude, with the experiment illustrated having the largest response observed. However, even with much lower switch responses stimulation of glucose release or portal pressure by AGEPC were not observed in retrograde perfusions.

The concentration-dependence of the glucose release stimulated by AGEPC indicates that the liver is affected significantly by the direction of perfusion flow (Fig. 5). The retrograde perfused liver is about 1000-fold less sensitive to AGEPC than the anterograde perfusion. The differential metabolic response may be attributed to the heterogeneous location of specific binding sites for AGEPC. To examine this possibility the results were compared with the glycogenolytic effects of agents

Fig. 4. Effect of *sn*-3-AGEPC on glucose output from liver perfused in the retrograde or anterograde direction

Livers were perfused in the anterograde direction between 0 and 35 min and 55 and 80 min, and in the retrograde direction between 35 and 55 min. $16:0$ -*sn*-3-AGEPC (1×10^{-9} M perfusate concentration) was infused between 45 and 47, and 65 and 67 min (■). Effluent was analysed for glucose and expressed as $\mu\text{mol/h per g}$ as described in the Materials and methods section. Results from one representative perfusion are shown.

Fig. 5. Effect of perfusion direction on the concentration-dependence of glucose release stimulated by *sn*-3-AGEPC

Livers were perfused and effluent analysed for glucose as described in the Materials and methods section. Between 35 and 37 min *sn*-3-AGEPC was infused to give the perfusate concentrations shown. The maximum increases in the rate of glucose output above basal are plotted as functions of the $\log_{10}$ [*sn*-3-AGEPC]. At least two perfusions were performed at each concentration; means $\pm$ S.E.M. are shown. ○, anterograde; ●, retrograde.

known to act directly at the level of the hepatocyte, or initially at some other liver cell type. Fig. 6 shows that the response of the liver to phenylephrine, an α -adrenergic agonist, is not affected by the direction of perfusate flow. In contrast, glucose release stimulated by latex particles shows a similar gradient of response as AGEPC, although the absolute magnitudes of these

Fig. 6. Effect of perfusion direction on the concentration-dependence of glucose release stimulated by phenylephrine or latex microspheres

Livers were perfused and glucose output was expressed as described in the legend to Fig. 5. At least two perfusions were performed for each concentration of phenylephrine or latex particles and shown as means $\pm$ S.E.M. □, Anterograde perfusion; ■ retrograde perfusion.

Table 2. Effect of *sn*-3-AGEPC on glucose release from cultured monolayers of hepatocytes or liver tissue slices

Each preparation was preincubation in Krebs saline prior to incubation in fresh saline in the absence or presence of AGEPC or phenylephrine as described in the text. Samples of medium were analysed for glucose content at 1, 5, 10, and 30 min, over which time glucose release was linear with incubation time. Data are expressed as means ($\pm$ S.E.M.) of duplicate incubations from two (monolayers) or three (slices) separate livers.

Addition	Glucose release from:	
	Hepatocyte monolayers (nmol/h per dish)	Tissue slices ($\mu\text{mol/h per g}$)
None	30.0 ± 8.1	13.5 ± 4.5
1×10^{-9} M-AGEPC	32.4 ± 9.4	13.1 ± 8.0
1×10^{-7} M-AGEPC	26.1 ± 5.1	9.2 ± 0.7
1×10^{-6} M-phenylephrine	75.0 ± 16.0	31.0 ± 3.7

responses differ. The retrograde perfused liver is about 5-fold less sensitive to latex particles as the anterograde perfusion. These results suggest that AGEPC may interact with the liver, at least initially, at the same site as phagocytosable particulate substances (i.e. the reticulo-endothelial cells), and not at the site of α -adrenergic agonist receptor binding (i.e. the hepatocytes).

The role of the reticuloendothelium in the biological response to AGEPC was also tested by measuring glucose release from cultured monolayers of hepatocytes

(endothelium free) and from suspensions of liver slices (non-perfused endothelium). Table 2 shows that the basal rates of glucose release were stimulated between 2 and 3-fold by the α -adrenergic agonist phenylephrine, but were not affected significantly by 1 or 100 mM-AGEPC. These results provide further evidence suggesting that the primary site of action of AGEPC in the liver is at the vascular endothelium.

DISCUSSION

This report presents visual and metabolic evidence which, in tandem, suggest that specific binding sites for platelet activating factor (*sn*-3-AGEPC) exist in the portal vasculature of the rat liver. Initial experiments designed to identify the location of *sn*-3-AGEPC binding in the liver indicate that the parenchyma, or non-vascular tissue, does not bind this compound, either specifically or non-specifically. This conclusion was reached from three lines of evidence. First, autoradiographs of this region do not show a density of silver grains above background levels. Second, exposure of the perfused livers to different concentrations of bovine serum albumin had no effect on the density of silver grains over the parenchyma. This was in contrast to the inhibitory effects of increasing concentrations of albumin on the density of silver grains over the portal sinusoids, and the glucose release in response to AGEPC. And last, the sensitivity of the liver to *sn*-3-AGEPC is reduced in retrograde compared with anterograde perfusions. Whereas this gradation of sensitivity contrasts with the response of the liver to agents which bind primarily to the hepatocyte membrane, it is similar to substances which interact with the reticuloendothelial system. These results corroborate previous reports which show that isolated hepatocyte suspensions, in contrast with the perfused liver, do not release glucose in response to exposure to *sn*-3-AGEPC (Buxton *et al.*, 1984; Fisher *et al.*, 1984; Mendlovic *et al.*, 1984). This lack of an effect may be due to loss or damage of hepatocyte receptors for AGEPC, as suggested previously (Fisher *et al.*, 1984). However, the observation that cultured monolayers of hepatocytes are insensitive to AGEPC indicates that specific receptors for AGEPC are not present in the hepatocyte plasma membrane.

Specific binding of a substance to a receptor is usually assessed by determining the displacement of binding of the labelled substance by an excess of the unlabelled substance. Binding of a substance is indicated as being non-specific when an excess of the unlabelled substance does not change the binding of the labelled substance. Specific binding can also be assessed by the induction of a biological response to the substance in question. Autoradiographs of liver slices exposed to [3 H]18:0-*sn*-3-AGEPC show that it binds to the first tissue with which it comes in contact. In anterograde perfusions this is the periportal side, and in retrograde perfusions this is the retrograde side of the liver. In retrograde perfusions, this binding appears to be non-specific, as the biological response is greatly reduced. Although our results do not identify the mechanism involved in this decreased sensitivity of the retrograde perfused liver, it may result from a titration of non-specific binding sites in the perivenous vasculature. Since the distribution of silver grains in the retrograde perfusion showed significant diffusion into

the parenchyma, it is most probable that the glycolytic response to high concentrations of AGEPC results from diffusion of ligand from the central venule, through the parenchyma and to the specific receptors in the portal sinusoids. However, the possibility cannot be excluded that the metabolic stimulation seen under these conditions arises from a non-specific interaction of micellar phospholipid with the hepatocyte membrane [the critical micellar concentration of *sn*-3-AGEPC is near 1 μ M (Kramp *et al.*, 1984)].

Evidence presented in the communication also suggests that the majority of binding of platelet activating factor within the portal vasculature is non-specific, as the pattern of silver grains was not qualitatively modulated by prior treatment of the liver with unlabelled *sn*-3-AGEPC, *sn*-1-AGEPC or U.66985. In contrast both the density of silver grains within the portal vasculature and glucose release stimulated by AGEPC could be inhibited by bovine serum albumin. The apparent $I_{0.5}$ for albumin of about 0.25% for both binding of AGEPC and inhibition of a biological response in rabbit platelets (Yamashita *et al.*, 1983) was similar to that seen in hepatic tissue (the present report). Further, since a biological response to *sn*-3-AGEPC was observed in the anterograde but not the retrograde perfusion, we conclude that specific binding sites are located in the portal vasculature, even though a large fraction of the observed binding is non-specific. This observation was not unexpected, as binding studies with platelets (Chesney *et al.*, 1983), and lung membrane preparations (Hwang *et al.*, 1985) also show that between 70 and 90% of [3 H]18:0-*sn*-3-AGEPC binding is non-specific (i.e. not displaceable with an excess of unlabelled *sn*-3-AGEPC).

Further evidence suggesting that AGEPC interacts specifically with the vascular endothelium comes from our observation that suspensions of liver slices, which contain both reticuloendothelial cells and hepatocytes, are unresponsive to AGEPC while retaining sensitivity to glycogenolytic agents which bind to the hepatocyte membrane. This result indicates that an intact and perfused vasculature is required for the ensuing biological responses of the liver to AGEPC. Parallel properties have been also reported for depolarizing gradients of potassium in the liver (Hill *et al.*, 1987). Both the AGEPC and the potassium responses have been suggested to result from vasoconstriction of the vasculature leading to localized ischaemia within the hepatic parenchyma (Buxton *et al.*, 1986a; Hill *et al.*, 1987). Although these reports showed that changes in the ATP/ADP ratio could not be detected in the perfused liver, we have recently shown that uric acid (and end-product of adenine nucleotide metabolism) release increases with increased perfusate concentrations of potassium, indicating that large but localized decreases in perfusate oxygen concentrations occur within the liver (Hill & Olson, 1987).

We propose that exogenous *sn*-3-AGEPC administered in the perfused rat liver binds to a discrete cell type within the portal vascular endothelium, and that this binding results in the major metabolic and haemodynamic consequences of exposure of the liver to AGEPC.

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REFERENCES

- Bergmeyer, H. -U., Bernt, E., Schmidt, F. & Stork, H. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), vol. 3, pp. 1196–1198, Academic Press, New York
- Buxton, D. B., Shukla, S. D., Hanahan, D. J. & Olson, M. S. (1984) *J. Biol. Chem.* **259**, 1468–1471
- Buxton, D. B., Fisher, R. A., Hanahan, D. J. & Olson, M. S. (1986a) *J. Biol. Chem.* **261**, 644–649
- Buxton, D. B., Hanahan, D. J. & Olson, M. S. (1986b) *Biochem. Pharmacol.* **35**, 893–897
- Campra, J. L. & Reynolds, T. B. (1982) in *The Liver: Biology and Pathobiology* (Arias, I., Popper, H., Schachter, D. & Shafritz, D. A., eds.), pp. 627–645 Raven Press, New York
- Charest, R., Prpic, V., Exton, J. H. & Blackmore, P. F. (1985) *Biochem. J.* **227**, 79–90
- Chesney, C. M., Pifer, D. D. & Huch, K. M. (1983) in *Platelet-Activating Factor* (Benveniste, J. & Arnoux, B., eds.), pp. 177–184, Elsevier Science Publishers, Amsterdam
- Fahimi, H. D. (1982) in *The Liver: Biology and Pathobiology* (Arias, I., Popper, H., Schachter, D. & Shafritz, D. A., eds.), pp. 495–506, Raven Press, New York
- Fisher, R. A., Shukla, S. D., DeBuysere, M. S., Hanahan, D. J. & Olson, M. S. (1984) *J. Biol. Chem.* **259**, 8685–8688
- Hill, C. E. & Olson, M. S. (1987) *Biochem. J.* **247**, 207–214
- Hill, C. E., Pryor, J. S., Olson, M. S. & Dawson, A. P. (1987) *J. Biol. Chem.* **262**, 6623–6627
- Hwang, S.-B., Lam, M.-H. & Shen, T. Y. (1985) *Biochem. Biophys. Res. Commun.* **128**, 972–979
- Ison, E. J. & Sheridan P. J. (1981) *Am. J. Med. Technol.* **47**, 38–42
- Jones, E. A. & Summerfield, J. A. (1982) in *The Liver: Biology and Pathobiology* (Arias, I., Popper, H., Schachter, D. & Shafritz, D. A., eds.), pp. 507–523, Raven Press, New York
- Kramp, W., Pieroni, G., Pinkard, R. N. & Hanahan, D. J. (1984) *Chem. Phys. Lipids* **35**, 49–62
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- McCuskey, R. S. (1966) *Am. J. Anat.* **119**, 455–478
- Mendlovic, F., Corvera, S. & Garcia-Sainz, J. A. (1984) *Biochem. Biophys. Res. Commun.* **123**, 507–514
- Miwa, M., Hill, C. E., Kumar, R., Sugatani, J., Olson, M. S. & Hanahan, D. J. (1987) *J. Biol. Chem.* **262**, 527–530
- Praaning-Van Dalen, D. P. & Knook, D. L. (1982) *FEBS Lett.* **141**, 229–232
- Scholz, R., Hansen, W. & Thurman, R. J. (1973) *Eur. J. Biochem.* **38**, 64–72
- Shukla, S. D., Buxton, D. B., Olson, M. S. & Hanahan, D. J. (1983) *J. Biol. Chem.* **258**, 10212–10214
- Widmann, J.-J., Cotran, R. S. & Fahimi, H. D. (1972) *J. Cell Biol.* **52**, 159–170
- Wisse, E. (1977) *J. Ultrastruct. Res.* **38**, 528–562
- Yamashita, M., Homma, H., Inoue, K. & Nojima, S. J. (1983) *Toxicol. Sci.* **8**, 177–188
- Yokota, S. (1985) *Anat. Rec.* **212**, 74–80

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