Evidence that Ca^{2+} fluxes and respiratory, glycogenolytic and vasoconstrictive effects induced by the action of platelet-activating factor and L- α -lysophosphatidylcholine in the perfused rat liver are mediated by products of the cyclo-oxygenase pathway

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The administration of ‘acetylglceryl ether phosphorylcholine’ (AGEPC, also known as platelet-activating factor) and L- α -lysophosphatidylcholine (LPC) to rat livers perfused with media containing 1.3 mM- Ca^{2+} was followed by a concentration-dependent efflux of Ca^{2+} from the liver. Near-maximal response was observed at 100 nM-AGEPC and 50 μM -LPC, and resulted in a net efflux of approx. 130 nmol of Ca^{2+} /g of liver. Onset of Ca^{2+} efflux occurred about 10 s after AGEPC and LPC administration, reached a maximum after about 50 s (the maximum rate of efflux was approx. 180 nmol/min per g) and thereafter decreased rapidly, and was sometimes followed by a much smaller influx of Ca^{2+} . Sequential infusions of AGEPC or LPC, and phenylephrine, indicate that each of these agents mobilizes Ca^{2+} from the same intracellular source. The efflux of Ca^{2+} was not observed in the presence of indomethacin or bromophenacyl bromide, or when the liver was perfused with low- Ca^{2+} -containing (25 μM) media. Other physiological responses, such as changes in respiration, glucose output and portal pressure, were also inhibited under these conditions. The results suggest that the Ca^{2+} -flux changes and other responses are mediated by prostaglandins produced and released within the liver, possibly by cell types other than hepatocytes.

INTRODUCTION

Apart from its established role in mediating inflammatory and allergic reactions, the phosphoglyceride AGEPC (platelet-activating factor) has been shown to have powerful effects on liver metabolism. In particular it has been reported that AGEPC elicits marked changes in vasoconstriction, oxygen consumption and glycogenolysis in the perfused rat liver (Shukla *et al.*, 1983; Buxton *et al.*, 1984, 1986; Fisher *et al.*, 1986). Although these effects of AGEPC on liver have been well characterized the mechanism by which such actions are mediated is not known. Of especial relevance are the findings that AGEPC does not elicit any respiratory and glycogenolytic effect in isolated hepatocytes (Fisher *et al.*, 1984). Although the reason for this is not clear, Buxton *et al.* (1986) and Fisher *et al.* (1986) have suggested that the respiratory and glycogenolytic responses in the perfused liver are secondary effects brought about by vasoconstriction of the vasculature system within the liver, and consequently a decreased oxygen supply to hepatocytes. This conclusion was partly based on a previous finding by these workers that the actions of AGEPC do not involve Ca^{2+} -flux movements in hepatocytes or the perfused liver, and hence that AGEPC induces glycogenolysis by a mechanism different from that of the Ca^{2+} -mobilizing agents (Fisher *et al.*, 1984; Buxton *et al.*, 1984).

In view of reports that AGEPC can lead to an increase in the cytosolic Ca^{2+} concentration in platelets (Hallam *et al.*, 1984), and can influence $^{45}\text{Ca}^{2+}$ movements in

cultured vascular endothelial cells (Brock & Gimbrone, 1986), and since the action of AGEPC has been shown to be dependent on extracellular Ca^{2+} (Buxton *et al.*, 1984, 1986), it seemed important to re-examine the issue of the action of AGEPC on whole-liver Ca^{2+} -flux changes. In this work we have used a Ca^{2+} -selective electrode to study the effects of AGEPC and lysophosphatidylcholine (LPC) administration on Ca^{2+} changes in the perfused rat liver. We also examined the effects of indomethacin (an inhibitor of cyclo-oxygenase) and bromophenacyl bromide (BPB, an inhibitor of phospholipase A_2) on the responses induced by AGEPC and LPC. We find that AGEPC and LPC each induce significant net Ca^{2+} -flux changes, and that these changes, together with the physiological responses of respiration, vasoconstriction and glycogenolysis, are all significantly inhibited by these inhibitors. These data provide strong evidence that cyclo-oxygenase products may be involved in mediating Ca^{2+} -flux changes and associated physiological responses by these agents.

METHODS

Male Wistar strain rats weighing 280–350 g and having free access to food were used for all experiments. The rats were anaesthetized with sodium pentobarbitone (50 mg/kg body wt.), and the livers were perfused at a flow rate of 35 ml/min with Krebs–Henseleit (1932) bicarbonate buffer containing 1.3 mM- CaCl_2 as described in Reinhart *et al.* (1982). Measurement of

Abbreviations used: AGEPC, ‘acetylglceryl ether phosphorylcholine’ (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine, platelet-activating factor); LPC, lysophosphatidylcholine; BPB, bromophenacyl bromide.

perfusate Ca^{2+} , oxygen and glucose concentrations was carried out exactly as described by Reinhart *et al.* (1982) and Altin & Bygrave (1985). Hepatic vasoconstriction was determined by measuring the portal pressure as reflected by the level of buffer in an open capillary (4 mm diameter) at the inflow to the liver. The basal portal pressure was 3.5 ± 0.3 mmHg; in this system an increase in pressure of 10 mmHg corresponded to a decrease in flow rate of approx. 13.7 ml/min at constant pressure. Stock solutions of AGEPC (0.7 mM) and LPC (17.5 mM) were made up in Krebs–Henseleit buffer containing 10% (w/v) fatty acid-free bovine serum albumin, and were infused into the portal vein at the appropriate rates by pump-driven infusion syringe. Indomethacin and BPB were dissolved in dimethyl sulphoxide and then administered by addition to the perfusion medium containing 0.1% albumin. The final concentration of dimethyl sulphoxide was $< 0.02\%$. Under these conditions neither dimethyl sulphoxide nor albumin had any significant effect on the action of the agents being studied.

AGEPC (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine), L- α -LPC (type I, from egg yolk), L- α -lysophosphatidylethanolamine (type I from egg yolk), L- α -phosphatidylcholine (type III-E from egg yolk), synthetic L- α -LPC (palmitoyl and stearyl forms), phenylephrine and indomethacin were all obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. BPB was obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A. All other reagents were of analytical grade.

RESULTS

Ca^{2+} -flux changes induced by the administration of AGEPC to the perfused rat liver

Although the action of AGEPC on hepatic vasoconstriction, respiration and glycogenolysis in the perfused rat liver has been studied in some detail (Shukla *et al.*, 1983; Buxton *et al.*, 1984, 1986), it appears that similar studies on the action of this substance on Ca^{2+} -flux movements in liver are lacking. Fig. 1 shows that the administration of AGEPC (100 nM) induces a rapid efflux of Ca^{2+} from the liver, as measured with the Ca^{2+} -selective electrode; also, the maximum rate and the total net amount of Ca^{2+} efflux are dependent on the concentration of AGEPC infused. The amount of Ca^{2+} efflux was close to maximal (120–140 nmol/g of liver; maximum rate of efflux was approx. 180 nmol/min per g of liver) at an AGEPC concentration of 100 nM. This amount is quantitatively similar to that induced by the α -adrenergic agonist phenylephrine (Reinhart *et al.*, 1982; Altin & Bygrave, 1985). Although the maximum rate and total amount of Ca^{2+} efflux are much less at the lower AGEPC concentrations (Fig. 1), measurable changes in perfusate $[\text{Ca}^{2+}]$ were induced at AGEPC concentrations as low as 0.1 nM (results not shown). These results suggest that AGEPC does elicit Ca^{2+} -flux movements in liver, and hence, contrary to the conclusions of Buxton *et al.* (1984), suggest that a mobilization of Ca^{2+} may be involved in triggering the glycogenolytic response that is induced by this agent.

Inhibition by indomethacin and BPB of AGEPC-induced Ca^{2+} fluxes and physiological responses

Of possible relevance to the mechanism of action of AGEPC is the finding that inhibitors of the arachidonate cascade can inhibit the glycogenolytic and vasocon-

Fig. 1. Ca^{2+} -flux changes induced by the administration of AGEPC to the perfused rat liver

Rat livers were perfused with Krebs–Henseleit bicarbonate medium containing 1.3 mM- Ca^{2+} as outlined in the Methods section. After a pre-perfusion period of 20 min, AGEPC was infused into the portal vein as indicated by the arrows. The continuous (—), broken (---) and dotted (····) traces represent Ca^{2+} -selective-electrode recordings of the perfusate Ca^{2+} concentration after the infusion of AGEPC at concentrations of 100, 10 and 1 nM respectively. Each trace is a representative obtained from four separate experiments.

strictive effects of AGEPC in the perfused rat liver (Mendlovic *et al.*, 1984). Because prostaglandins have been implicated in the transport of Ca^{2+} in some systems (see, e.g., Karmazyn, 1986; Fukuo *et al.*, 1986), it was decided to explore the possibility that these products of cyclo-oxygenase activity may be involved in mediating also the Ca^{2+} -flux changes (and other physiological responses) induced by AGEPC. The data in Figs. 2(a), 2(b), 2(c) and 2(d) show respectively the changes in perfusate concentrations of Ca^{2+} , oxygen and glucose, and also the change in portal pressure induced by the administration of 10 nM-AGEPC to the perfused liver. The data in Fig. 2 also show the effect of a 15 min pre-administration of indomethacin (20 μM). The results provide evidence of a marked ($> 80\%$) inhibition of all four AGEPC-induced responses by 20 μM -indomethacin. This strongly suggests that cyclo-oxygenase products, presumably prostaglandins, are involved in mediating the Ca^{2+} -flux changes and the other three physiological responses that are observed. It is noteworthy that a 15 min pre-infusion of BPB (50 μM) also inhibited the responses to a similar extent (results not shown). However, it is relevant to point out that, whereas indomethacin did not affect the responses induced by administration of phenylephrine, in this instance BPB inhibited the action of phenylephrine by approx. 50% (not shown).

Comparison of the effects of LPC with those of AGEPC

For several reasons it seemed relevant to study the effect of LPC in the perfused rat liver. Firstly, the basic molecular structures of AGEPC and LPC are similar. Secondly, LPC can be produced intracellularly and extracellularly by mechanisms including the action of phospholipase A_2 on phosphatidylcholine, and in plasma (by the action of the enzyme phosphatidylcholine-choles-

Fig. 2. Effect of AGEPC administration on net Ca²⁺-flux changes, oxygen consumption, glucose output and portal pressure, and the inhibition of these responses by indomethacin

Rat livers were perfused with Krebs–Henseleit medium containing 1.3 mM-Ca²⁺. The perfusate Ca²⁺ and oxygen concentrations were monitored continuously with the Ca²⁺-selective electrode and oxygen electrode respectively. At the indicated times the portal pressure was recorded, and samples of the effluent perfusate were collected for determination of glucose. Other details are outlined in the Methods section. After a pre-perfusion period of 20 min, AGEPC (10 nM) was infused into the portal vein for 10 min, as indicated by the arrows. The continuous curves in (a), (b), (c) and (d) show the AGEPC-induced change in the perfusate Ca²⁺, oxygen and glucose concentrations and portal pressure respectively. The dotted curves in (a), (b), (c) and (d) show the corresponding changes induced by AGEPC in experiments in which indomethacin (20 μM) was pre-administered for 15 min (beginning at 5 min of perfusion) and thereafter for the remainder of the experiment. In each instance the Ca²⁺ and oxygen traces are representative of between four and six independent experiments. The results for glucose concentration and portal pressure are means of four separate experiments; S.E.M. values have been omitted for clarity, but ± 0.04 mM for glucose, and ± 0.7 mmHg for portal pressure, was never exceeded.

terol acyltransferase), where the LPC is believed to be transported by albumin for uptake and utilization by cells (Feilding *et al.*, 1972; Burrier & Brecher, 1986). Moreover, the above studies indicated an involvement of

Fig. 3. Effect of LPC administration on net Ca²⁺-flux changes, oxygen consumption, glucose output and portal pressure, and the inhibition of these responses by indomethacin

The continuous curves in (a), (b), (c) and (d) show the changes in the perfusate Ca²⁺, oxygen and glucose concentrations and portal pressure respectively induced by the administration of LPC (50 μM) to the perfused rat liver. In each instance the corresponding dotted curve represents the result obtained in the presence of 20 μM-indomethacin. Liver perfusions and other experimental details, except that, where indicated, 50 μM-LPC was used instead of AGEPC, were exactly as described in the legend to Fig. 2.

the arachidonate cascade in the mechanism of action of AGEPC.

Preliminary experiments suggested that, in the concentration range 1–100 μM, the magnitude of the responses induced by LPC was correspondingly similar to those induced by AGEPC at concentrations in the range 0.1–100 nM. Moreover, the changes in the concentrations of perfusate Ca²⁺, oxygen and glucose and the increase in portal pressure induced by the administration of 50 μM-LPC are shown in Figs. 3(a), 3(b), 3(c) and 3(d) respectively. The data indicate clearly that LPC (50 μM) induces significant changes in all four parameters studied. Although the magnitude of the Ca²⁺-flux response induced by LPC was concentration-dependent and was only just detectable at 1–5 μM-LPC (results not shown), the response induced was near-maximal at 50 μM-LPC. In this instance LPC induced a

Fig. 4. Effect of LPC administration on oxygen consumption, and the dose-response curve of the effect of LPC on hepatic portal pressure

Rat livers were perfused with Krebs-Henseleit bicarbonate medium containing 1.3 mM- Ca^{2+} . After a pre-perfusion period of 15 min, LPC was administered for a period of 5 min (arrows). The curves in (a) represent the oxygen response obtained from separate experiments in which the concentration of LPC administered was 10 μM ($\cdots$), 25 μM (---) or 50 μM (—). Each result is a representative of at least three independent experiments. (b) shows the dose-response curve for the effect of LPC on the maximum change in portal pressure that is induced during the first 5 min of LPC administration in experiments of the type shown in (a). Each point represents the mean $\pm$ S.E.M. for three separate pressure measurements conducted at each concentration of LPC.

net efflux of Ca^{2+} (100–150 nmol/g of liver; maximum rate of efflux approx. 170 nmol/min per g of liver), followed by a smaller extent of Ca^{2+} influx (see Fig. 3a). The general shape of the oxygen response changed considerably with increasing concentrations of LPC. In the concentration range 1–10 μM , LPC stimulated oxygen uptake in a transient manner, whereas, at concentrations of around 20 μM and above, the oxygen response was biphasic, showing first a stimulation and then an inhibition of oxygen uptake (see Fig. 4a). At LPC concentrations of 1–10 μM , glucose output was sustained for the duration of PLC infusion (results not shown). However, at concentrations above about 20 μM , the maximum rate of glucose output increased rapidly, to high values initially, and then returned rapidly to near-basal values even while the LPC was still being infused (see, e.g., Fig. 3c). The data in Fig. 3(d) show that LPC also induced an increase in portal pressure. This increase was sustained for the period of LPC administration and was concentration-dependent (see Fig. 4b).

Comparison of the effects induced by AGEPC (see Fig. 2 of the present work; also Buxton *et al.*, 1984, 1986) with those induced by LPC (Fig. 3) suggests that the main differences are in the dose-response curve (LPC active at a 1000-fold higher concentration than AGEPC), and in the ability of LPC to induce a more sustained effect on vasoconstriction and inhibition of oxygen uptake (cf. Figs. 3 and 4) at near-maximal concentrations of each agent. The responses induced by LPC are also inhibited by a 15 min pre-administration of indomethacin (Fig. 3) or 50 μM -BPB (results not shown). As with AGEPC (Fig. 2), these results suggest that cyclo-oxygenase products are involved in eliciting the responses induced by LPC (Fig. 3).

The source of Ca^{2+} mobilized by AGEPC and LPC

Our finding that AGEPC and LPC administration to the perfused rat liver results in an efflux of Ca^{2+} (Figs. 1 and 2) raises the question of the source of this Ca^{2+} . In

order to study this, a higher concentration of AGEPC (100 nM) was used in the following experiments to ensure near-maximal Ca^{2+} efflux. The data in Fig. 5(a) show that a 4 min pre-administration of 100 nM-AGEPC results in abolition of the Ca^{2+} -efflux response induced by a subsequent infusion of 5 μM -phenylephrine. Conversely, Fig. 5(b) shows that a prior administration of 5 μM -phenylephrine, which is known to lead to a depletion of the hormone-sensitive pool of Ca^{2+} (Reinhart *et al.*, 1982), results in an almost total inhibition of the Ca^{2+} efflux induced by subsequent infusion of AGEPC. These results suggest that the major component of Ca^{2+} mobilized by AGEPC originates from the same intracellular source as the Ca^{2+} that is mobilized by phenylephrine. Hence, since it is thought that in liver phenylephrine mobilizes Ca^{2+} from the endoplasmic reticulum in hepatocytes (reviewed by Reinhart *et al.*, 1984; Williamson *et al.*, 1985; Exton, 1985), it would appear that the main component of the Ca^{2+} efflux response induced by AGEPC (Figs. 1, 2a and 5) is due also to an efflux of Ca^{2+} from this source.

Two further features of the Ca^{2+} mobilization induced by AGEPC may be noted. Firstly, although AGEPC and phenylephrine appear to mobilize Ca^{2+} from the same intracellular source, the Ca^{2+} mobilization induced by AGEPC is inhibitable by 20 μM -indomethacin, whereas that induced by phenylephrine is not inhibitable by this substance (Fig. 5c). This would suggest that cyclo-oxygenase products are involved in the release of Ca^{2+} from hepatocytes. Secondly, the mobilization of Ca^{2+} by phenylephrine is unaffected by lowering of $[\text{Ca}^{2+}]$ in the perfusion medium from 1.3 mM (physiological) to 25 μM ; however, the mobilization of Ca^{2+} induced by AGEPC is abolished under these conditions (Fig. 5d). This suggests that an adequate extracellular $[\text{Ca}^{2+}]$ is crucial for the mobilization of Ca^{2+} by AGEPC to occur, and therefore may explain the failure of Buxton *et al.* (1984) to detect any AGEPC-induced Ca^{2+} fluxes in their system, in which the extracellular Ca^{2+} concentration was only 50 μM . Essentially identical results were

Fig. 5. Effect of sequential additions of AGEPC and phenylephrine on the Ca²⁺-efflux response

Rat livers were perfused with Krebs-Henseleit bicarbonate medium, and the perfusate Ca²⁺ concentration was continuously monitored with the Ca²⁺-selective electrode as previously described. At 20 min and 25 min of perfusion, respectively, AGEPC (100 nM) and phenylephrine (Phen; 5 μ M) were administered as indicated by the arrows. The resulting Ca²⁺-flux response is shown in (a). The Ca²⁺-flux response induced when the order of AGEPC and phenylephrine administration was reversed is shown in (b). AGEPC and phenylephrine infusions in (c) were similar to (a), except that 20 μ M-indomethacin was administered at 5 min of perfusion and thereafter for the remainder of the experiment. (d) shows the Ca²⁺ response induced by the infusion of AGEPC for 4 min, followed by phenylephrine for 4 min as indicated; however, in this instance the Ca²⁺ concentration of the perfusion medium was changed from 1.3 mM- to 25 μ M-Ca²⁺ at 20 min of perfusion. Each trace is a representative obtained from three separate experiments.

obtained when 50 μ M-LPC was used instead of AGEPC (results not shown).

DISCUSSION

The first major conclusion from this work is that, provided that there is an adequate concentration of

external Ca²⁺, AGEPC is able to induce significant Ca²⁺-flux changes in the perfused rat liver. This offers an explanation why in previous reports AGEPC did not lead to any significant Ca²⁺-flux changes in this system (Buxton *et al.*, 1984). As a consequence of this, current models for the mechanism by which AGEPC elicits its effects in liver (see, e.g., Buxton *et al.*, 1984, 1986; Fisher *et al.*, 1986) may need to be revised.

The second major point is that LPC administration to the perfused rat liver elicits responses, including Ca²⁺-flux changes, oxygen uptake, glucose output and hepatic vasoconstriction, which are similar to those induced by AGEPC. This previously has not been reported. Because it has been demonstrated that LPC can induce cell lysis at high concentrations (Weltzien, 1979), it was important to establish that any irreversible damage to hepatocytes under these conditions had not occurred. Our observations that, at concentrations up to 50 μ M, repeated short administrations (< 5 min) of LPC lead to a repetition of the responses, and that a subsequent administration of the α -agonist phenylephrine induces a normal type of response if administered after a suitable rest period (results not shown), strongly suggest that under these conditions little irreversible damage had occurred.

It is possible that LPC may interact with receptors on the plasma membrane, perhaps through low-affinity interaction with receptors for AGEPC. Alternatively, the action of LPC may be brought about by a less specific effect on the ion-permeability properties of the plasma membrane (Weltzien, 1979). It is noteworthy however, that similar concentrations of phosphatidylcholine and L- α -lysophosphatidylethanolamine from egg yolk were without effect, whereas synthetic L- α -LPC (palmitoyl or stearoyl forms) were both active in the present system (results not shown). Despite obvious similarities between the responses induced by LPC and those induced by AGEPC, some differences are apparent. These include a difference in the dose-response [cf. Fig. 4(b) of the present work with Fig. 4 in Fisher *et al.* (1986)], a more sustained vasoconstrictive effect and inhibition of oxygen uptake during LPC administration (Fig. 3), and a decreased susceptibility to desensitization upon subsequent administrations, by comparison with AGEPC (results not shown).

An important feature of the responses induced by AGEPC and LPC is their sensitivity to inhibition by BPB and indomethacin. This argues strongly for an involvement of phospholipase A₂ and the production of prostaglandins in eliciting the responses. Of potential relevance to this work are the findings that cultured Kupffer and endothelial cells from rat liver sinusoids produce significant amounts of prostaglandins and other eicosanoids when stimulated by agents such as AGEPC, arachidonic acid, phorbol ester and zymosan (Birmelin & Decker, 1984; Dieter *et al.*, 1986; Schlayer *et al.*, 1986). By contrast, hepatocytes are more likely to be involved in the degradation rather than in the synthesis of prostaglandins (Decker, 1985; Tran-Thi *et al.*, 1986). In the light of these considerations, the present results suggest that the responses induced by the administration of AGEPC and LPC to the liver may be due to an interaction of these agents with cell types other than the parenchymal cells. The possible involvement of cell types other than hepatocytes in the action of AGEPC previously had been proposed by Mendlovic *et al.* (1984).

In essence, these findings could well explain the inability of AGEPC to induce any glycogenolytic effect in isolated hepatocytes (Fisher *et al.*, 1984).

Consistent with this notion also are the findings (Charest *et al.*, 1985) that AGEPC does not stimulate inositol trisphosphate formation, or an increase in cytoplasmic $[Ca^{2+}]$ in isolated hepatocytes. Moreover, our present observation that AGEPC does not induce any sizeable amount of Ca^{2+} efflux from the perfused liver at low perfusate Ca^{2+} (Fig. 5d) supports these findings. On the other hand, this does not support the observations by Shukla *et al.* (1983) and Fisher *et al.* (1984) that AGEPC stimulates the breakdown of phosphatidylinositol 4,5-bisphosphate in isolated hepatocytes. A further implication of the present work is that extracellular Ca^{2+} is important in the production of prostaglandins. Since phospholipase A_2 is a Ca^{2+} -dependent enzyme, and since treatment of Kupffer cells and endothelial cells with the Ca^{2+} -ionophore A23187 is sufficient to activate the arachidonate cascade (Dieter *et al.*, 1986), it seems that the much decreased ability of AGEPC to stimulate responses in liver at low perfusate $[Ca^{2+}]$ can be explained.

In regard to the mechanism of action of AGEPC, Buxton *et al.* (1986) and Fisher *et al.* (1986) have proposed that the glycogenolytic effects of AGEPC are brought about indirectly by a decreased oxygen supply induced by vasoconstriction. Because both AGEPC and LPC induce a release of Ca^{2+} from the same source as that mobilized by the α -agonist phenylephrine, it seems to us more likely that glycogenolysis is triggered by the release of this Ca^{2+} , perhaps by the interaction of cyclo-oxygenase products with hepatocytes through receptors that are themselves linked to the breakdown of phosphoinositides.

On the basis of the evidence presented, we propose the following model to explain the observed AGEPC- and LPC-induced responses. Clearly, central to this is that AGEPC and LPC elicit various responses in the perfused rat liver by a mechanism that involves the cyclo-oxygenase pathway. The data are consistent with a general model in which AGEPC and LPC in liver interact primarily on eicosanoid-producing cells, such as Kupffer cells and endothelial cells. The nature of this interaction is unknown at present, but could involve the breakdown of phosphoinositides in these cells such that there is an increase in cytosolic $[Ca^{2+}]$ and an activation of phospholipase A_2 , as has been reported for the action of AGEPC on platelets (Billah & Lapetina, 1983). The eicosanoid products released, in particular the prostaglandins, then could interact both with hepatocytes, producing Ca^{2+} -flux changes, a stimulation of oxygen uptake and glycogenolysis, and also with the vasoactive cells that are involved in affecting hepatic vasoconstriction. The nature of the interaction between the prostaglandins and the parenchymal cells of the liver is not known, but may itself involve the breakdown of phosphoinositides by a mechanism similar to that which occurs in the action of Ca^{2+} -mobilizing hormones (Creba

et al., 1983; Berridge, 1984; Charest *et al.*, 1985). We acknowledge, however, that a direct proof for the existence of this mechanism awaits further investigation.

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