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Prostaglandin $F_{2\alpha}$ and the thromboxane A_2 analogue ONO-11113 stimulate Ca^{2+} fluxes and other physiological responses in rat liver

Further evidence that prostanoids may be involved in the action of arachidonic acid and platelet-activating factor

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The administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and the thromboxane A_2 analogue, ONO-11113, to rat livers perfused with media containing either 1.3 mM- or 10 μM-Ca^{2+} was followed by a stimulation of Ca^{2+} efflux, changes in O_2 uptake and glucose output, and increase in portal pressure. The responses elicited by 5 μM-$PGF_{2\alpha}$ were similar to those induced by the α-adrenergic agonist phenylephrine. At both 1.3 mM and 10 μM extracellular Ca^{2+}, $PGF_{2\alpha}$ induced Ca^{2+} efflux (70–90 nmol/g of liver), probably from the same source as that released by phenylephrine. Prostaglandin D_2 (5 μM) and prostaglandin E_2 (5 μM) also induced responses, but these were generally much smaller (< 30%) than those induced by $PGF_{2\alpha}$. Similarly to vasopressin and other Ca^{2+}-mobilizing hormones, $PGF_{2\alpha}$ also interacted synergistically with glucagon (and cyclic AMP) in stimulating Ca^{2+} influx both in the perfused liver and in isolated hepatocytes. By comparison with phenylephrine and $PGF_{2\alpha}$, ONO-11113 was much more potent in inducing vasoconstriction, and, at concentrations of 10–200 nM, induced a different pattern of changes in Ca^{2+} flux, respiration and glycogenolysis. There was first a rapid efflux of Ca^{2+} (45–60 nmol/g of liver), followed by a smaller Ca^{2+} influx, and a further release of Ca^{2+} (approx. 90 nmol/g of liver) when ONO-11113 was removed. Respiration was first stimulated but then markedly inhibited. At concentrations less than 5 nM, ONO-11113 induced a sustained stimulation of O_2 uptake and a more prolonged efflux of Ca^{2+}, with less Ca^{2+} efflux occurring upon the removal of the agent. Glycogenolysis followed a pattern which was similar to the Ca^{2+} response. Co-administration of glucagon did not potentiate Ca^{2+} influx by ONO-11113, but the action of ONO-11113 was inhibited (50%) by a few minutes' prior administration of 10 nM-vasopressin. The vasoconstrictive action of ONO-11113 was synergistically potentiated by the co-administration of phenylephrine. Since the actions of arachidonic acid, platelet-activating factor and lysophosphatidylcholine in liver were recently found to be cyclo-oxygenase-sensitive, the results provide strong evidence that at least $PGF_{2\alpha}$ and thromboxane A_2 may be involved in mediating the action of these agents.

INTRODUCTION

Recent work suggests that eicosanoids, metabolites generally associated with an inflammatory response, may be produced by different cell types from liver (e.g. Kupffer and endothelial cells in culture) after exposure to an appropriate stimulus (Birmelin & Decker, 1984; Dieter *et al.*, 1986; Schlayer *et al.*, 1986). Although there are strong indications that eicosanoids may be taken up and metabolized by hepatocytes (Decker, 1985; Tran-Thi *et al.*, 1986), and that specific receptors may exist on the surface of hepatocytes for the binding or transport of leukotrienes (Uehara *et al.*, 1983) and prostaglandins (Smigel & Fleischer, 1974, 1977; Okamura & Terayama, 1977; Okumura *et al.*, 1987), as yet it is unclear as to whether these receptors have a specific role in regulating liver function. There are reports which suggest that the administration of prostaglandins of the E-series to the perfused rat liver (DeRubertis *et al.*, 1974) and to isolated hepatocytes (Bronstad & Christoffersen, 1981; Brass *et al.*, 1984; Brass & Garrity, 1985) can modulate the effects of other hormones such as glucagon and adrenaline by inhibiting the hormone-induced accumulation of cyclic AMP. It appears, nonetheless, that relatively little is known about the mechanism of action of these and other prostanoids in liver.

Products of cyclo-oxygenase activity have been implicated in the action of arachidonic acid (Dieter *et al.*, 1987*a*,*b*; Altin & Bygrave, 1987*b*) and of platelet-activating factor (Mendlovic *et al.*, 1984; Buxton *et al.*, 1984; Garcia-Sainz & Hernandez-Sotomayor, 1985; Altin *et al.*, 1987) in the perfused rat liver. Such action includes a stimulation of Ca^{2+}-flux changes, O_2 uptake, glucose output and an increase in portal pressure (see references above). In order to establish which prostaglandins might be involved in mediating these responses, we have examined in this work the effects of administering prostaglandin D_2 (PGD_2), prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and a stable analogue of thromboxane A_2 (ONO-11113) to the perfused rat liver.

Abbreviation used: PG, prostaglandin.

The data obtained provide new information about the mechanism of action of prostanoids in liver. It appears that the action of $PGF_{2\alpha}$ is mediated by a mechanism similar to that of other Ca^{2+}-mobilizing hormones, whereas the action of ONO-11113 seems likely to involve a different mechanism. The possible involvement of these prostanoids in mediating some of the actions of arachidonic acid and platelet activating factor in liver is discussed.

METHODS

Liver perfusions

Male Wistar-strain rats of weight 280–350 g and fed *ad libitum* were anaesthetized with sodium pentobarbitone (50 mg/kg body wt.). After dissection, the livers were perfused in a non-recirculating mode with Krebs–Henseleit (1932) bicarbonate medium equilibrated with O_2/CO_2 (19:1) and containing 1.3 mM-Ca^{2+} as described previously (Reinhart *et al.*, 1982). After a 15–20 min pre-perfusion period, hormones and prostanoids were administered at the specified concentration by an appropriate infusion with a pump-driven syringe. The concentrations of O_2 and Ca^{2+} in the outflowing perfusate was monitored continuously with a Clark-type oxygen electrode and a Ca^{2+}-selective electrode (Radiometer F2112), respectively, as described by Reinhart *et al.* (1982) and Altin & Bygrave (1985). Where indicated, portal pressure was determined by measuring the level of buffer in an open glass capillary (2 mm diameter) connected to the inflow canula into the portal vein; the basal portal pressure was 3.4 ± 0.3 mmHg. Also, for some experiments, appropriate samples of the perfusate were collected for glucose determination by the glucose oxidase method (Reinhart *et al.*, 1982).

Stock solutions of phenylephrine, vasopressin, glucagon and $PGF_{2\alpha}$ were made up in Krebs–Henseleit medium, whereas those of PGD_2, PGE_2 and thromboxane-A_2 analogue ONO-11113 were made in dimethyl sulphoxide. The final concentration of dimethyl sulphoxide when used as a solvent was less than 0.05% (v/v). All solutions were stored frozen at −20 °C.

Preparation of hepatocytes

Hepatocytes were prepared by a modification of the methods described by Berry & Friend (1969) and Barritt *et al.* (1981). After 10 min of low-Ca^{2+} perfusion, the liver was perfused for 6–8 min with medium containing 1.3 mM-Ca^{2+} plus collagenase (0.03%, w/v). The liver was dispersed and then incubated for a further 10 min in the same medium in a 250 ml conical flask kept at 37 °C and shaken at 100 cycles/min in a water-bath shaker. Cell clumps were removed by filtering with a nylon mesh (0.2 mm grid), and the cell suspension was washed twice by gentle centrifugation (50 *g* for 1 min), aspirating away the supernatant, and resuspending of the cell sediment in ice-cold Krebs–Henseleit bicarbonate medium. A final wash was carried out with complete minimum essential Eagle's medium containing 1.3 mM free Ca^{2+} and 20 mM-Hepes buffer, pH 7.4, but without Phenol Red. The cells were resuspended in this medium (30 mg of cells/ml), dispensed into 1 ml portions, and incubated for 15 min at 37 °C before addition of any agonist. All incubations were carried out with constant shaking and gassing with O_2/CO_2 (19:1). For all experiments cells were used within 1 h of preparation. Cell viability, as judged by the exclusion of Trypan Blue, always exceeded 90%.

For determination of the total cell calcium, at the appropriate time the cells were immediately placed in an Eppendorf tube and centrifuged for 10–15 s to pellet the cells. The supernatant was quickly removed and the cell protein was precipitated with ice-cold 2 M-$HClO_4$ to extract calcium. The precipitate was pelleted, and a suitable sample of the supernatant was used for calcium determination by atomic-absorption spectroscopy.

Chemicals and materials

PGD_2, PGE_2, $PGF_{2\alpha}$, phenylephrine, [Arg^8]vasopressin, glucagon, dibutyryl 3′,5′-cyclic AMP (hereafter 'cyclic AMP'), collagenase (type IV), fatty-acid-free bovine serum albumin and the glucose assay kit (510-A) were all obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. The stable analogue of thromboxane A_2, 9,11-epithio-11,12-methano-thromboxane A_2, was generously given by Ono Pharmaceutical Co., Higashiku, Osaka, Japan. Percoll and density-marker beads were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Ca^{2+}-selectrode membranes (F2112) and filling solution S43316 were obtained from Radiometer, Copenhagen, Denmark. Other chemicals used were of analytical grade.

RESULTS

Preliminary experiments indicated that, of the three prostaglandins tested, $PGF_{2\alpha}$ was by far the most active. The data in Fig. 1(*a*) show the Ca^{2+}-flux changes induced by the administration of $PGF_{2\alpha}$ (5 μM) to the liver perfused with media containing either 10 μM (dotted curve) or 1.3 mM (continuous curve) free Ca^{2+}. The administration of $PGF_{2\alpha}$ induces a significant efflux of Ca^{2+} from the liver (70–90 nmol/g of liver) which is taken up when the agent is removed. Although the time of onset of Ca^{2+} efflux is slower (approx. 15 s), and the net amount of Ca^{2+} efflux is less, the overall pattern of the response is similar to that induced by 2 μM-phenylephrine (see Fig. 1*a*, and Reinhart *et al.*, 1982; Altin & Bygrave, 1985). The administration of $PGF_{2\alpha}$ is also accompanied by a stimulation of whole-tissue respiration and glucose output, and an increase in portal pressure, as shown in Figs. 1(*b*), 1(*c*) and 1(*d*) respectively. By contrast, the administration of PGE_2 induced a relatively small effect (< 30%) by comparison with $PGF_{2\alpha}$, and the administration of PGD_2 induced responses that were only just detectable (see Fig. 1). It is noteworthy that the action of $PGF_{2\alpha}$ was similar to that of phenylephrine, in that both agents induced a Ca^{2+} efflux and less-sustained physiological responses even when the perfusate Ca^{2+} concentration was lowered to 10 μM.

Intracellular source of Ca^{2+} mobilized by $PGF_{2\alpha}$

The data in Fig. 1(*a*) suggest that $PGF_{2\alpha}$ may induce its effects by inducing a release of Ca^{2+} from intracellular stores. It was therefore of interest to establish whether the Ca^{2+} release induced by $PGF_{2\alpha}$ originates from the same intracellular source as that mobilized by other Ca^{2+}-mobilizing agents, such as phenylephrine (see, e.g., Reinhart *et al.*, 1982, 1984; Exton, 1985; Williamson *et al.*, 1985). The data in Fig. 2(*a*) show that Ca^{2+} efflux induced by the administration of $PGF_{2\alpha}$ is much

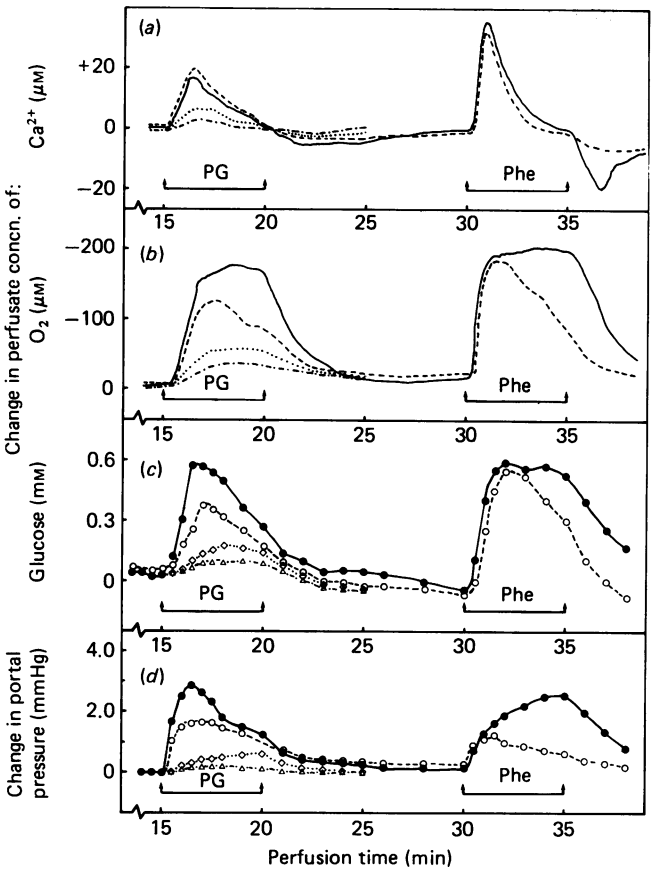

Fig. 1. Effect of PGD_2, PGE_2 and $PGF_{2\alpha}$ administration on Ca^{2+}-flux changes, O_2 consumption, glucose output and portal pressure in the perfused rat liver

Livers were perfused with Krebs–Henseleit bicarbonate medium containing 1.3 mM-Ca^{2+} (——) or 10 μM-Ca^{2+} (– – –; lowered from 1.3 mM to 10 μM at 10 min of perfusion and thereafter for the remainder of the experiment) as detailed in the Methods section. Separate experiments were conducted in which, after a pre-perfusion period of 15 min, PGD_2 (·–·–, △) PGE_2 (····, ◇) or $PGF_{2\alpha}$ (——, ●; – – –, ○) (each at 5 μM) was infused for 5 min as indicated by the arrows. Then 20 min later phenylephrine (Phe; 2 μM) was infused as shown. The induced Ca^{2+} and O_2 responses, as monitored with the Ca^{2+}-sensitive electrode and oxygen electrode respectively, are shown in (*a*) and (*b*). The associated glucose response and changes in portal pressure are shown in (*c*) and (*d*) respectively, where each point is the result of a measurement carried out at the indicated time (see the Methods section for further details). For clarity, the data for the effects of PGD_2 and PGE_2 at 10 μM perfusate Ca^{2+} were not included, but all responses induced were smaller than those induced at 1.3 mM-Ca^{2+}. Each trace is a representative of those obtained from three separate experiments. The data for glucose and portal pressure represent mean values obtained from the three experiments; S.E.M. values were omitted for clarity, however, ±0.03 mM for glucose and ±0.2 mmHg for portal pressure was never exceeded. For clarity, the data obtained with PGD_2 and PGE_2 are not presented beyond the 25 min time point.

decreased after the depletion of the intracellular hormone-sensitive pool of Ca^{2+} by phenlephrine. Similarly, the administration of $PGF_{2\alpha}$ diminishes the subsequent

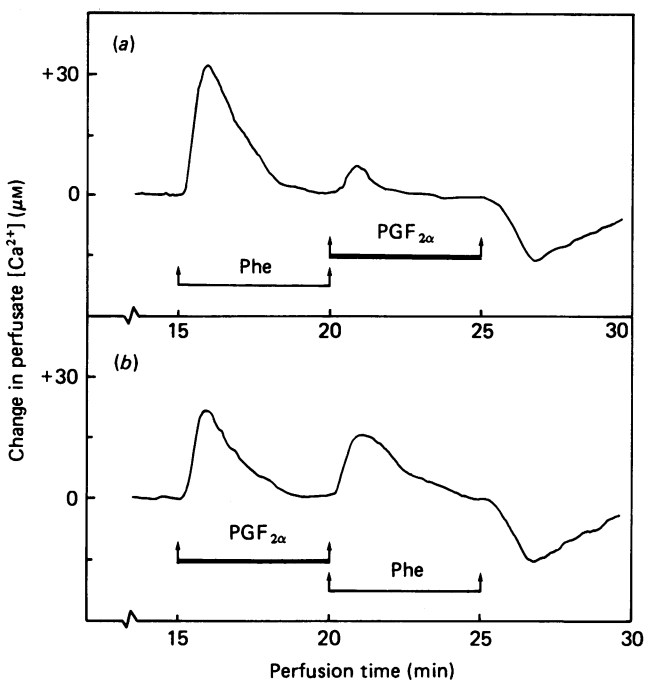

Fig. 2. Effect of sequential infusion of $PGF_{2\alpha}$ and phenylephrine on Ca^{2+} efflux

Rat livers were perfused with Krebs–Henseleit bicarbonate medium containing 1.3 mM-Ca^{2+}. At 15 min of perfusion phenylephrine (Phe; 5 μM) and then $PGF_{2\alpha}$ (5 μM) were each infused for 5 min as indicated. The Ca^{2+} response induced is shown in (*a*); (*b*) shows the Ca^{2+} response obtained from a similar experiment in which the order of $PGF_{2\alpha}$ and phenylephrine administration was reversed. Each trace is a representative of three experiments performed independently.

efflux of Ca^{2+} induced by phenylephrine. These results therefore suggest that $PGF_{2\alpha}$ mobilizes Ca^{2+} from the same intracellular source as does phenylephrine.

Effect of glucagon co-administration on the Ca^{2+}-flux changes induced by PGD_2, PGE_2 and $PGF_{2\alpha}$

To determine whether the cyclic-AMP-producing hormone glucagon could alter the pattern of Ca^{2+}-flux change induced by the prostaglandins (compare with the effect of glucagon on the responses induced by other Ca^{2+}-mobilizing agents: Morgan *et al.*, 1983; Mauger *et al.*, 1985; Poggioli *et al.*, 1986; Altin & Bygrave, 1986, 1987*a*), experiments were conducted in which both glucagon and prostaglandins were co-administered. The data in Fig. 3(*b*) show the Ca^{2+} responses induced by the co-administration of glucagon (10 nM) with PGD_2, PGE_2 or $PGF_{2\alpha}$ (each at 5 μM) 4 min later. By comparison with Fig. 3(*a*), which shows a small efflux of Ca^{2+} induced by the administration of $PGF_{2\alpha}$ alone, the co-administration of glucagon (10 nM) and 5 μM-$PGF_{2\alpha}$ resulted in a large stimulation of Ca^{2+} influx. The amount of Ca^{2+} influx that was induced (approx. 1000 nmol of Ca^{2+}/g of liver) with these concentrations of glucagon and $PGF_{2\alpha}$ is quantitatively similiar to that induced by the co-administration of glucagon with 2 μM-phenylephrine under otherwise identical experimental conditions (see Fig. 3 and Table 1). It is noteworthy that, when co-administered with glucagon, $PGF_{2\alpha}$ induced

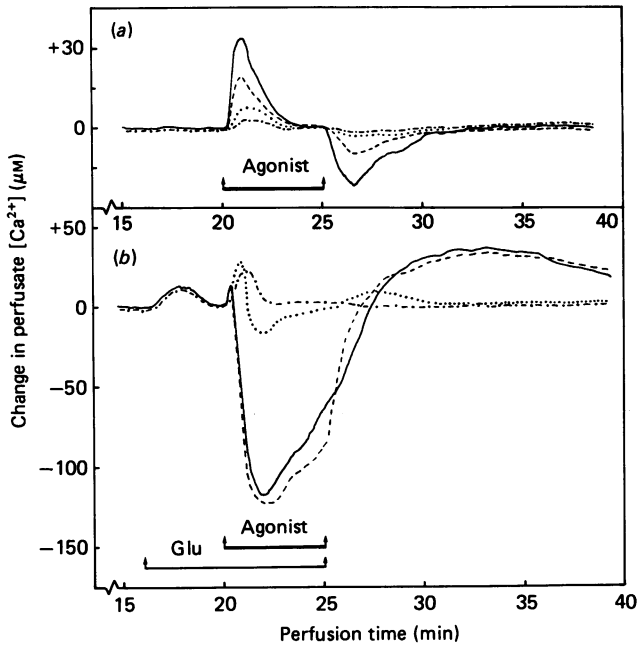

Fig. 3. Comparison of the Ca^{2+}-flux response induced by the co-administration of glucagon with PGD_2, PGE_2, $PGF_{2\alpha}$, or with phenylephrine

Rat livers were perfused with Krebs–Henseleit bicarbonate medium containing 1.3 mM-Ca^{2+}, and the perfusate Ca^{2+} concentration was monitored continuously with a Ca^{2+}-selective electrode. The traces in (*a*) represent the Ca^{2+} response obtained from separate experiments in which phenylephrine (2 μM; ——) or PGD_2 (–·–·), PGE_2 (····) or $PGF_{2\alpha}$ (– – – –), each at 5 μM, was infused for the time indicated by the arrows. Similarly, the Ca^{2+} response obtained in separate experiments in which the infusion of phenylephrine, PGD_2, PGE_2 and $PGF_{2\alpha}$ was preceded by a 4 min infusion of 10 nM-glucagon (Glu), which was then co-administered with each agent is shown in (*b*). At this concentration, the administration of glucagon induced a small efflux of Ca^{2+} during the first 3–4 min (see Fig. 3*b*), but did not itself induce any net stimulation of Ca^{2+} influx when administered for the 9 min period (results not shown). Essentially identical results (not shown) were obtained when 200 μM-cyclic AMP was administered instead of glucagon. Each trace is a representative of three or four independent experiments.

Table 1. Stimulation of net Ca^{2+} influx in the perfused rat liver by the co-administration of glucagon with PGD_2, PGE_2, $PGF_{2\alpha}$ or phenylephrine

The Table gives the net amount and maximum rate of Ca^{2+} influx induced by the co-administration of 10 nM-glucagon and PGD_2, PGE_2 and $PGF_{2\alpha}$, or phenylephrine. The maximum rate of influx was calculated from the maximum deflection of the pen in the Ca^{2+} trace, and the net amount of Ca^{2+} influx from the area above the curve in the Ca^{2+} influx response. Liver perfusions and other details are exactly as described in the legend to Fig. 3(*b*). Each value represents the mean ± S.E.M. obtained from three to five independent experiments.

	Ca^{2+} influx	
Addition of glucagon plus	Net amount (nmol/g of liver)	Maximum rate (nmol/min per g of liver)
PGD_2 (5 μM)	None detected	No change
PGE_2 (5 μM)	52 ± 8	23 ± 5
$PGF_{2\alpha}$: (0.2 μM)	112 ± 9	50 ± 4
(1 μM)	470 ± 65	157 ± 22
(5 μM)	1105 ± 130	324 ± 46
Phenylephrine (2 μM)	1030 ± 128	320 ± 38

Table 2. Effect of the co-addition of glucagon with PGD_2, PGE_2 or $PGF_{2\alpha}$ (each at 5 μM), or with phenylephrine (2 μM), on the total calcium content of isolated hepatocytes

Cells were incubated with glucagon for 4 min and then for a further 5 min with the agent indicated below. After harvesting, the cells were then assayed for calcium content. See the Methods section and the text for further details. Each value represents the mean ± S.E.M. obtained from five or six experiments performed independently. No significant change was induced by PGD_2 or PGE_2, or when glucagon alone was present.

Additions	Total calcium content (nmol/g wet wt. of cells)
Control (no additions)	1800 ± 180
Glucagon plus $PGF_{2\alpha}$	2564 ± 170
Glucagon plus phenylephrine	2592 ± 190

significant influx of Ca^{2+} even at a concentration of 0.2 μM (Table 1). By contrast, the co-administration of glucagon only slightly potentiated the Ca^{2+} response induced by PGD_2 and PGE_2 (each at 5 μM) (see Fig. 2 and Table 1), a result consistent with the smaller effect of these prostaglandins on Ca^{2+}-flux movements, glycogenolysis and respiration, when administered alone (see Fig. 1). In all instances, similar results were obtained when 200 μM-cyclic AMP was used in place of glucagon.

Cell type and the intracellular sink for the Ca^{2+} influx

The change in the total cell calcium content induced by the administration of the different prostaglandins and phenylephrine, with or without glucagon, is shown in Table 2. By comparison with Fig. 3 and Table 1, the increase in calcium content (approx. 800 nmol/g wet wt. of cells) induced by the addition of these agents to isolated hepatocytes shows a similar pattern to that which is induced in the perfused rat liver. Because the proportion of parenchymal to non-parenchymal cells is increased in this preparation as compared with the intact liver, the data suggest that the principal action of $PGF_{2\alpha}$ is on hepatocytes themselves. Also, the similarity of the response induced by $PGF_{2\alpha}$ to that induced by phenylephrine when each is co-administered with glucagon provides further evidence for a common mechanism of action.

The large stimulation of Ca^{2+} influx induced by the co-administration of glucagon and $PGF_{2\alpha}$ raises the question of the intracellular sink for this Ca^{2+}. To examine this, liver subfractionation studies were conducted to deter-

mine the location of the Ca^{2+} taken up after the co-administration of glucagon and $PGF_{2\alpha}$ to the perfused liver. Although in these experiments glucagon and $PGF_{2\alpha}$ were co-administered for only 5 min (as in Fig. 3), the studies were carried out exactly as described previously for locating the intracellular sink for the Ca^{2+} influx after the co-administration of glucagon and vasopressin (see Altin & Bygrave, 1986). The data indicate that, whereas the calcium content of plasma-membrane- and endoplasmic-reticulum-enriched fractions increased by 30–50%, the calcium content of the fractions enriched in mitochondria increased approx. 10-fold, each with respect to control fractions obtained from livers treated with glucagon only (results not shown). This suggests that the mitochondria are the sink for the Ca^{2+} influx that is observed, a feature similar to that reported for the Ca^{2+} uptake induced by the co-administration of glucagon with vasopressin (Altin & Bygrave, 1986).

Effect of ONO-11113, a stable analogue of thromboxane A_2, on Ca^{2+}-flux changes, respiration, glycogenolysis and vasoconstriction

Since the response induced by each of the prostaglandins examined in this work could not account for some of the responses induced by platelet-activating factor and lysophosphatidylcholine, it was decided to study the possible action of the cyclo-oxygenase metabolite thromboxane A_2 on physiological responses in liver. Because thromboxane A_2 is unstable, we used the stable analogue ONO-11113. Fig. 4 shows that the administration of 20 nM-ONO-11113 to the liver perfused with media containing 1.3 mM-Ca^{2+} induces rapid biphasic Ca^{2+}-flux changes. The infusion of ONO-11113 induced a rapid efflux of Ca^{2+} during the first 40–50 s, corresponding to 45–60 nmol/g of liver. The onset of Ca^{2+} efflux occurred at about 8 s, and the maximum rate of efflux (approx. 200 nmol/min per g of liver) was reached after about 30 s of ONO-11113-administration. This was followed by a spontaneous influx of Ca^{2+} (30–40 nmol/g of liver), which reached a maximum rate of around 55 nmol Ca^{2+}/min per g of liver after approx. 1 min of ONO-11113 administration. These effects were accompanied first by a small transient stimulation, and then by a rapid and sustained inhibition, of O_2 uptake (Fig. 4*b*). Also, there was a transient pulse of glucose release, and a marked and sustained increase in portal pressure (see Figs. 4*c* and 4*d*). The removal of ONO-11113 was followed by a transient efflux of Ca^{2+} (approx. 90 nmol/g of liver), a relief of respiratory inhibition, with perhaps a slight stimulation of O_2 uptake, a transient stimulation of glucose output, and a return to basal portal pressure (see Fig. 4). Although dependent also on the concentration of ONO-11113 used (results not shown), all these responses were considerably decreased (inhibited by 30–70%) by perfusion with media containing low (10 μM) Ca^{2+} (see broken curves in Fig. 4). This suggests that the presence of extracellular Ca^{2+} is important for the full expression of the action of ONO-11113.

Whereas the pattern of responses shown in Fig. 4 was typical of the responses induced by ONO-11113 at concentrations of 10–200 nM, a different pattern of Ca^{2+}-flux change, respiratory and glycogenolytic responses was induced by ONO-11113 at concentrations below about 5 nM. For example, Fig. 5(*a*) shows that 2.5 nM-ONO-11113 induces a more prolonged Ca^{2+} efflux and

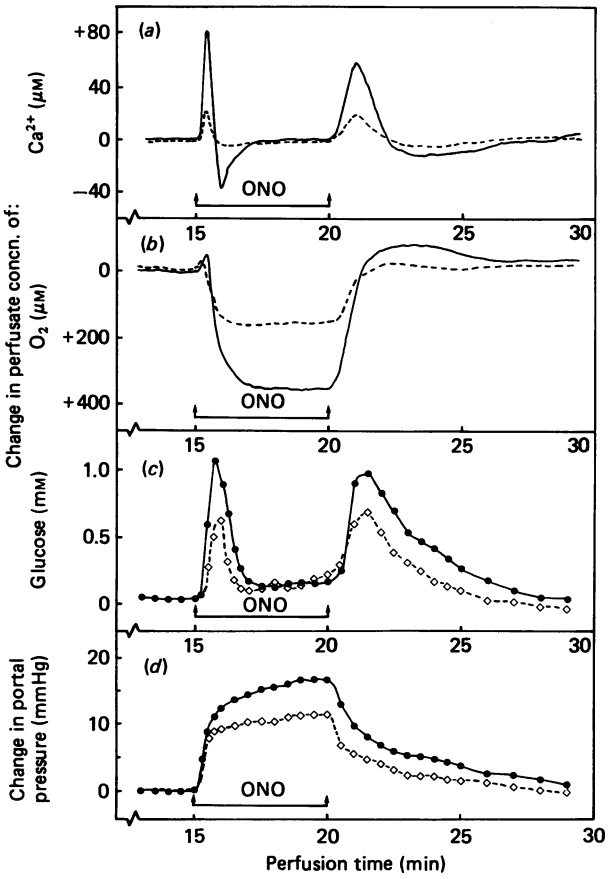

Fig. 4. Effect of the administration of the thromboxane A_2 analogue, ONO-11113, on Ca^{2+}-flux changes, O_2 consumption, glucose output and portal pressure in the perfused rat liver

Livers were perfused with Krebs–Henseleit bicarbonate medium containing 1.3 mM-Ca^{2+} (——) or 10 μM-Ca^{2+} (----; lowered from 1.3 mM to 10 μM at 10 min of perfusion and thereafter for the remainder of the experiment) as detailed in the Methods section. After a pre-perfusion period of 15 min, ONO-11113 (20 nM) was infused for 5 min as indicated by the arrows. The induced changes in perfusate Ca^{2+}, O_2 and glucose, and portal pressure, are shown in (*a*), (*b*), (*c*) and (*d*) respectively. Other details are exactly as described in the legend to Fig. 1. Each trace is a representative of those obtained from three to five separate experiments. The glucose data represent mean values; S.E.M. values were omitted for clarity, however, $\pm$0.04 mM was never exceeded.

glucose output during its administration. In this instance a net stimulation of Ca^{2+} influx occurs only when the agent is removed. Comparison with Fig. 4 shows that ONO-11113 induced a moderate increase in portal pressure and a sustained stimulation of O_2 uptake (see Fig. 5*b*). Also, the stimulation of Ca^{2+} efflux and glucose output, upon the removal of ONO-11113 and coincident with the relief of vasoconstriction, were considerably decreased at the lower ONO-11113 concentrations, when the increase in portal pressure stimulated by ONO-11113 was much less (Fig. 5). This suggests that severe vasoconstriction and associated inhibition of respiration induced during the administration of higher ONO-11113 concentrations may be interfering with the quantitative output of Ca^{2+} and glucose (cf. Figs. 4 and 5).

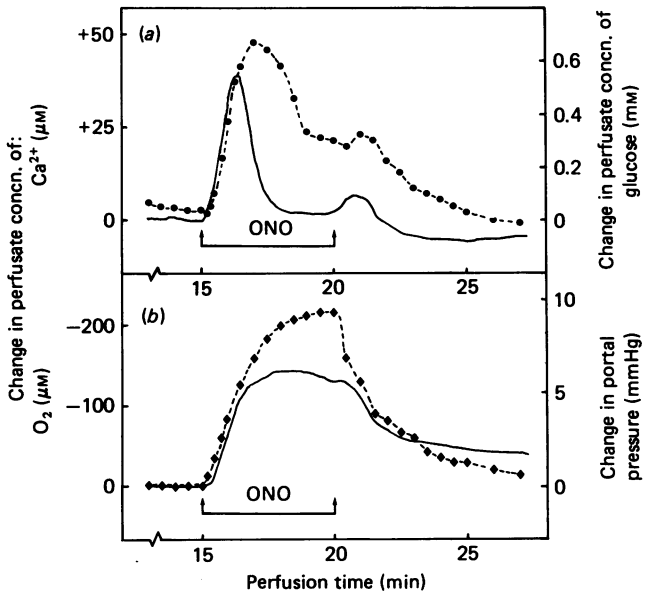

Fig. 5. Effect of the administration of low concentrations of ONO-11113 on Ca^{2+} fluxes, O_2 uptake, glucose output and portal pressure

Rat livers were perfused exactly as described in the legend to Fig. 4, except that media containing 1.3 mM-Ca^{2+} were used throughout all experiments. The Ca^{2+} (——) and glucose (●) responses induced by a 5 min administration of ONO-11113 (2.5 nM, arrowed) are shown in (*a*). Similarly, the associated changes in O_2 uptake (——) and portal pressure (◆) are shown in (*b*). Other details are exactly as in the legend to Fig. 4.

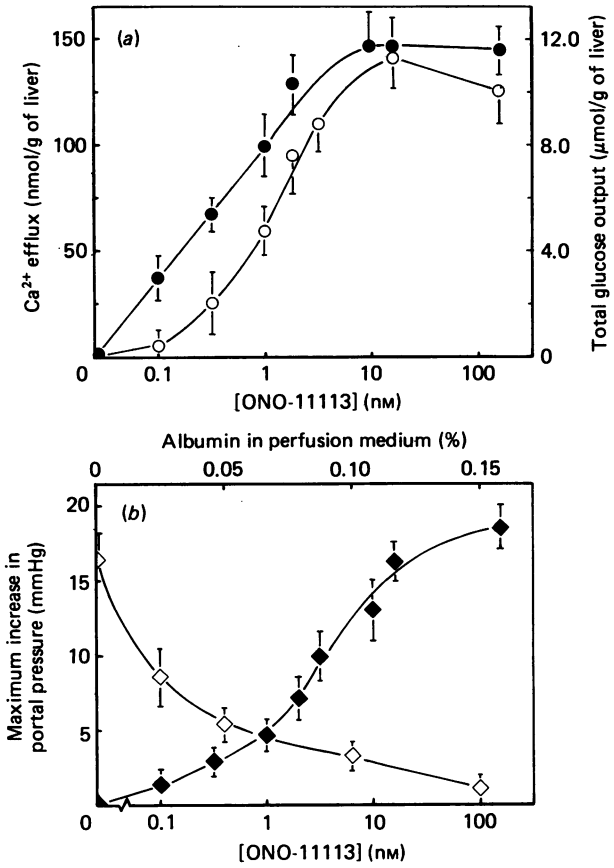

Fig. 6. Dose–response for the stimulation of Ca^{2+} efflux, glucose output and portal pressure by ONO-11113

Perfusion details are exactly as described in the legend to Fig. 4, except that Krebs–Henseleit bicarbonate medium containing 1.3 mM-Ca^{2+} was used throughout all experiments, and the concentration of ONO-11113 administered was varied as indicated. In (*a*) each point (●) represents the total amount of Ca^{2+} efflux induced both during and 5 min after a 5 min ONO-11113 administration. Similarly each point (○) represents the total glucose output induced by ONO-11113 during the same period. In (*b*) each point (◆) represents the maximum increase in portal pressure that is induced during the 5 min administration of ONO-11113 at the specified concentration. Data for each point within a curve were obtained from separate experiments conducted at the indicated concentration of ONO-11113. Also in (*b*) the points (◇) represent the maximum increase in portal pressure that is induced during a 5 min administration of 20 mM-ONO-11113, but for which the specified amount of bovine serum albumin was pre-administered for 5 min and thereafter continuously administered. Each point is the mean ± S.E.M. of three to six experiments performed independently.

Dose–response for the action of ONO-11113 on physiological responses, and the effect of including bovine serum albumin in the perfusion medium

Because at concentrations above 10 nM ONO-11113 induced responses both during and immediately after its administration, it seemed important to take into account both these effects in determining the dose–response. Thus Fig. 6(*a*) shows the total Ca^{2+} efflux and glucose output induced during, and 5 min after, a 5 min administration of different concentrations of ONO-11113. The increases in portal pressure induced by the administration of different concentrations of ONO-11113 are shown in Fig. 6(*b*). From the shape of the curves it is clear that ONO-1111 has very potent effects on Ca^{2+} efflux, glucose output and vasoconstriction. Near-maximal effects for each of these responses were induced at 20 nM-ONO-11113 (Fig. 6). It was noted, however, that the co-administration of bovine serum albumin had a pronounced moderating effect on the action of ONO-11113 (see Fig. 6*b*). The inclusion of 0.03% bovine serum albumin in the perfusion medium resulted in a shift in the dose–response curve by approximately an order of magnitude to the right (results not shown).

Effect of glucagon co-administration on the Ca^{2+} response induced by ONO-11113

The observation that ONO-11113 induces a mobilization of Ca^{2+} led us to investigate whether its mechanism of action, at least with respect to Ca^{2+} mobilization, is similar to that of other Ca^{2+}-mobilizing agents. The data in Fig. 7(*a*) show that, under conditions where ONO-11113 is presumed to release intracellular Ca^{2+} (see Fig. 7), the co-administration of glucagon does not lead to any stimulation of Ca^{2+} influx (cf. glucagon plus $PGF_{2\alpha}$ and glucagon plus phenylephrine, Fig. 3). By contrast, despite the vasoconstriction induced by ONO-11113 under these conditions, phenylephrine was still capable of stimulating Ca^{2+} influx (Fig. 7*a*). This suggests that the mechanism of action of ONO-11113 in eliciting Ca^{2+}

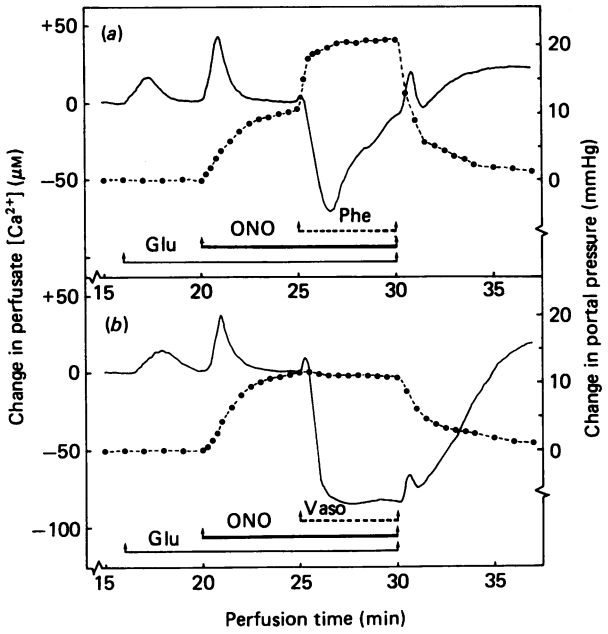

Fig. 7. Effect of the co-administration of glucagon with ONO-11113, phenylephrine or vasopressin on Ca^{2+} flux changes and portal pressure

Rat livers were perfused with Krebs–Henseleit bicarbonate medium containing 1.3 mM-Ca^{2+}, and the perfusate Ca^{2+} concentration was monitored continuously with a Ca^{2+}-selective electrode. At 16 min of perfusion, glucagon (Glu; 10 nM) and 4 min later ONO-11113 (2.5 nM), and 5 min later phenylephrine (Phe; 2 μM) were all co-infused until 25 min of perfusion. The Ca^{2+} trace (——) and the changes in portal pressure (●) are shown in (*a*). Similarly, the Ca^{2+} and portal-pressure responses obtained in separate experiments in which vasopressin (Vaso; 10 nM) was infused in place of phenylephrine are shown in (*b*). Essentially identical results were obtained when 200 μM-cyclic AMP was administered instead of glucagon (results not shown). Data for each Ca^{2+} trace and portal pressure are representative of three experiments performed independently.

efflux is different from that of phenylephrine and $PGF_{2\alpha}$. Because the amount of Ca^{2+} influx induced by phenylephrine was decreased to approx. 40% in the presence of ONO-11113, it would seem that ONO-11113-induced vasoconstriction was interfering with phenylephrine-induced Ca^{2+} influx. Although the prior administration of glucagon had only a slight inhibitory effect on the vasoconstrictive action of ONO-11113, subsequent infusion of phenylephrine resulted in a synergistic stimulation of portal pressure (Fig. 7*a*). This synergism occurred also when ONO-11113 and phenylephrine was administered without glucagon (results not shown). That vasoconstriction interfered with Ca^{2+} influx is also suggested by the fact that, when vasopressin was administered in place of phenylephrine, there was no synergistic increase in portal pressure, and Ca^{2+} influx was more sustained (Fig. 7*b*). It was noteworthy that, in the absence of glucagon, a few minutes' prior administration of 10 nM-vasopressin alone inhibited all responses induced by a subsequent co-administration of ONO-11113 (results not shown).

DISCUSSION

The first major finding from this work relates to the ability of $PGF_{2\alpha}$ to induce Ca^{2+}-flux changes, a stimulation of respiration and glycogenolysis, and vasoconstriction when administered to the liver perfused with media containing either high (1.3 mM) or low (10 μM) concentrations of Ca^{2+}. It appears that such action of $PGF_{2\alpha}$ in liver has not yet been reported.

This work also reveals that the action of $PGF_{2\alpha}$ has a number of features in common with that of the so-called Ca^{2+}-mobilizing hormones. Firstly, $PGF_{2\alpha}$ appears to release Ca^{2+} from the same intracellular source as that mobilized by phenylephrine, since a pre-administration of phenylephrine abolishes the Ca^{2+} efflux induced by a subsequent administration of $PGF_{2\alpha}$. Secondly, $PGF_{2\alpha}$ and glucagon (or cyclic AMP) interact synergistically in stimulating Ca^{2+} influx both in the perfused rat liver and in isolated hepatocytes. This influx is dependent on the concentration of $PGF_{2\alpha}$, with 5 μM-$PGF_{2\alpha}$ inducing essentially the same amount of Ca^{2+} influx as that induced by a near-maximal concentration of phenylephrine. Thirdly, the mitochondria are the intracellular sink for the Ca^{2+} taken up, as determined by the Ca^{2+} content of fractions obtained by a Percoll-density gradient subfractionation of liver after co-administration of glucagon and $PGF_{2\alpha}$. These findings also suggest that $PGF_{2\alpha}$ elicits its effects by interacting with parenchymal cells in liver.

The action of Ca^{2+}-mobilizing agents is known to involve a mobilization of Ca^{2+} that occurs as a consequence of a receptor-mediated breakdown of phosphoinositides and the generation of second messengers (Creba *et al.*, 1983; Joseph *et al.*, 1984; Berridge, 1984; Charest *et al.*, 1985). To our knowledge there are no reports of $PGF_{2\alpha}$ inducing phosphatidylinositide breakdown in liver parenchyma. It is noteworthy, however, that $PGF_{2\alpha}$ has been shown to have growth-factor-like action on neonatal-rat hepatocytes in culture (Armato & Andreis, 1983), to stimulate phosphatidylinositol turnover and accumulation of 1,2-diacylglycerol in resting 3T3 Swiss cells (Macphee *et al.*, 1984), to stimulate the production of inositol phosphates in cultured rat mesangial cells (Mene *et al.*, 1987), and to stimulate phosphatidylinositol 4,5-bisphosphate hydrolysis and a mobilization of intracellular Ca^{2+} in bovine luteal cells (Davis *et al.*, 1987). These actions of $PGF_{2\alpha}$ are consistent with our present results, which suggest that in liver the action of $PGF_{2\alpha}$ is mediated by a mechanism similar to that of other Ca^{2+}-mobilizing agents.

Although PGE_2 was considerably more effective than PGD_2 in inducing Ca^{2+} movements and a stimulation of respiration and glycogenolysis, both prostaglandins in our system induced only small effects on these responses, especially when compared with the magnitude of those induced by $PGF_{2\alpha}$. Because hepatocytes have been shown to possess receptors for PGD_2 and PGE_2, and since both of these prostaglandins are known to be produced by non-parenchymal liver cells (e.g. Kupffer cells) in culture (Decker, 1985), it would seem that the principal role of these prostaglandins in regulating liver metabolism may be different from that of the Ca^{2+}-mobilizing hormones. In this regard it may be relevant that E-series prostaglandins have been reported to inhibit cyclic-AMP-dependent and cyclic-AMP-independent hormone-stimulated glycogenolysis in isolated hepato-

cytes (Brass & Garrity, 1985). In our system a prior administration of glucagon or cyclic AMP led to a small but significant stimulation of Ca^{2+} efflux upon subsequent administration of PGD_2 or PGE_2 (see Fig. 3). The mechanism for this is not clear.

Our data indicate that the thromboxane analogue ONO-11113 is very active in its ability to stimulate physiological responses in the perfused rat liver (see Fig. 5). Such action was also reported by Fisher *et al.* (1987), who carried out their studies with the structurally different analogue U-46619. A significant difference in action between these two compounds appears to be that the potency of ONO-11113 is at least an order of magnitude greater than that of U-46619 (cf. Fig. 6 of present work with Fig. 2 of Fisher *et al.*, 1987). Similarly, the principal differences between the action of ONO-11113 and $PGF_{2\alpha}$, as far as we can judge, are the greater potency of ONO-11113 in inducing vasoconstriction, a different pattern of Ca^{2+}, respiratory and glycogenolytic response, and the inability of glucagon to potentiate Ca^{2+} influx induced by ONO-11113. The manner in which bovine serum albumin is able to moderate the action of ONO-11113 is not known, but could involve the binding of ONO-11113 to albumin or some interference effect of the albumin on the interaction of ONO-11113 with the thromboxane receptor. In support of this is our observation that the bovine serum albumin effect could be overcome by increasing the concentration of ONO-11113 (results not shown).

As pointed out by Fisher *et al.* (1987), it is possible that the respiratory and glycogenolytic effects of thromboxane analogues in liver occur secondarily to vasoconstriction, especially in view of the fact that severe vasoconstriction can be expected to lead to ischaemia within the liver tissue. Such action may well explain the observed inhibition of tissue respiration, and consequently a stimulation of glycogenolysis, by an increase in AMP within hepatocytes themselves (Hers, 1976; Hems & Whitton, 1980). It is also noteworthy, however, that whereas no Ca^{2+}-flux changes were reported by Fisher *et al.* (1987), who studied the action of U-46619, the action of ONO-11113 is associated with marked Ca^{2+}-flux movements in liver perfused with media containing a physiological concentration (1.3 mM) of Ca^{2+} (see Figs. 5 and 6). We cannot exclude the possibility that some component of the Ca^{2+} movement induced by ONO-11113 may result from the direct action of ONO-11113 on smooth-muscle cells within the hepatic vasculature or other cell types within the liver. Alternatively, this may also involve a release of Ca^{2+} from pools within the extracellular space that is associated with the parenchymal cells of the liver (Claret-Berthon *et al.*, 1977). Owing to the magnitude of the Ca^{2+} efflux that is induced (Fig. 6*a*), and the fact that the mobilization of such appears to be temporally related to the stimulation of glycogenolysis (see Figs. 4 and 5), we believe that the major component of the Ca^{2+} efflux induced by ONO-11113 is due to a mobilization of Ca^{2+} from hepatocytes, or at least must be in close communication with the cytoplasmic free Ca^{2+} concentration of hepatocytes. It may be possible that the Ca^{2+} release induced by ONO-11113 occurs as a consequence of hypoxia and the associated increases in intracellular AMP (or other metabolites) within hepatocytes, similar to the reported stimulation of glycogenolysis which can occur under these conditions. Such an effect of hypoxia on Ca^{2+} efflux from hepatocytes has yet to be demonstrated, however. Our measurements of the time of onset of Ca^{2+} efflux and vasoconstriction suggest that the onset of Ca^{2+} efflux may occur just before vasoconstriction. It is clear, therefore, that the mechanism by which ONO-11113 and perhaps other thromboxane A_2 analogues stimulate Ca^{2+}-flux changes in the perfused rat liver will need to be clarified, before the glycogenolytic action of these agents can be attributed solely to their ability to induce hypoxia.

A final major point arising from this work is that the results presented support our proposal that the action of arachidonic acid (Dieter *et al.*, 1987*a*, *b*; Altin & Bygrave, 1987*b*), platelet-activating factor and lysophosphatidylcholine (Altin *et al.*, 1987) are mediated by eicosanoids produced and released by different cell types within the liver. The responses induced by $PGF_{2\alpha}$, including its interaction with glucagon, are similar to those induced by the administration of arachidonic acid (Altin & Bygrave, 1987*b*). On the other hand, conditional upon the concentrations used, the responses induced by ONO-11113 share many features with those induced by the administration of platelet-activating factor and lysophosphatidylcholine (see Altin *et al.*, 1987). Since the actions of arachidonic acid, platelet-activating factor and lysophosphatidylcholine are all sensitive to inhibition by indomethacin, it would seem that the principal action of arachidonic acid in liver is mediated mainly by the generation of $PGF_{2\alpha}$, whereas the actions of platelet-activating factor and lysophosphatidylcholine are mediated mainly by the generation of thromboxane A_2. We do not eliminate the possibility that these agents may, in addition, have some direct action on liver cells. However, because arachidonic acid is the precursor of both $PGF_{2\alpha}$ and thromboxane A_2, and the responses induced by arachidonic acid and ONO-11113 are different (cf. this work with Dieter *et al.*, 1987*b*; Altin & Bygrave, 1987*b*), it appears that, in the main, exogenously administered arachidonic acid is metabolized by different pathways, or perhaps by cell types different from those that are responsible for the activation of cyclo-oxygenase activity by platelet-activating factor and lysophosphatidylcholine (Altin *et al.*, 1987*a*). According to this model, $PGF_{2\alpha}$ released from such cells would interact with hepatocytes, eliciting physiological responses by a mechanism similar to that of Ca^{2+}-mobilizing hormones (i.e. by interacting with receptors linked to the breakdown of phosphoinositides). The vasoactive response seen when $PGF_{2\alpha}$ is administered might be attributable also to an interaction with smooth-muscle cells. Also, the thromboxane A_2 produced could interact with vasoactive cells and possibly hepatocytes, inducing vasoconstriction and a stimulation of other responses by mechanisms which are not yet fully understood. This interpretation presumes that the concentrations of prostaglandins used in these experiments are comparable with those that may be generated locally within the liver. To our knowledge no estimate of local concentrations has yet been made, however.

In this work we have related the action of arachidonic acid and platelet-activating factor to that of $PGF_{2\alpha}$ and of thromboxane A_2. Clearly these will be only a fraction of the total spectrum of eicosanoids which could be generated in liver and modulate its metabolism, consequent to the exposure to agents such as arachidonic acid, lysophosphatidylcholine and platelet-activating factor. We therefore consider that the full effects of these agonists in liver can only be understood when the

composite effects, interactions and concentrations of all the active metabolites produced are taken into account.

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