Phosphatidic acid and arachidonic acid each interact synergistically with glucagon to stimulate Ca^{2+} influx in the perfused rat liver

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The administration of phosphatidic acid 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, O_2 uptake and glucose output. The responses elicited by 100 μM -phosphatidic acid were similar to those induced by the α -adrenergic agonist phenylephrine. Contrary to suggestions that phosphatidic acid acts like a Ca^{2+} -ionophore, no net influx of Ca^{2+} was detected until the phosphatidic acid was removed. Sequential infusions of phenylephrine and phosphatidic acid indicate that the two agents release Ca^{2+} from the same intracellular source. The co-administration of glucagon (or cyclic AMP) and phosphatidic acid, and also of glucagon and arachidonic acid, led to a synergistic stimulation of Ca^{2+} uptake of the liver, a feature similar to that observed after the co-administration of glucagon and other Ca^{2+} -mobilizing hormones [Altin & Bygrave (1986) *Biochem. J.* 238, 653–661]. A notable difference, however, is that the synergistic stimulation of Ca^{2+} uptake induced by the co-administration of glucagon and arachidonic acid was inhibited by indomethacin, whereas that induced by glucagon and phosphatidic acid, or glucagon and other Ca^{2+} -mobilizing agents, was not. The results suggest that the synergistic action of glucagon and arachidonic acid in stimulating Ca^{2+} influx is mediated by prostanoids, but that of glucagon and phosphatidic acid is evoked by a mechanism similar to that of Ca^{2+} -mobilizing agents.

INTRODUCTION

Until recently, neither phosphatidic acid nor arachidonic acid has been implicated in the mechanism of receptor-induced Ca^{2+} mobilization, at least in the perfused rat liver, although each may be produced either directly or indirectly as a consequence of a receptor-induced activation of phospholipase C and phospholipase A_2 respectively. Exogenously added phosphatidic acid has been reported to increase cellular Ca^{2+} and to evoke various physiological responses in cells (e.g. Barritt *et al.*, 1981; Ohsako & Deguchi, 1981). These effects often have been explained in terms of the findings that phosphatidic acid displays Ca^{2+} -ionophoretic properties in some systems (Tyson *et al.*, 1976; Putney *et al.*, 1980; Serhan *et al.*, 1981; Harris *et al.*, 1981). This has led to suggestions that phosphatidic acid may be involved in mediating the influx of Ca^{2+} induced by Ca^{2+} -mobilizing hormones (e.g. Putney *et al.*, 1980; Harris *et al.*, 1981). More recently, however, Moolenaar *et al.* (1986) have reported that, in human A341 carcinoma cells, phosphatidic acid elicits its effects by triggering the breakdown of phosphoinositides and releasing Ca^{2+} from intracellular stores, and not by stimulating Ca^{2+} influx. On the other hand, although it is well established that arachidonic acid can be metabolized to eicosanoids in many cell types (for reviews, see Decker, 1985; Needleman *et al.*, 1986), other reports suggest that arachidonic acid also has a potency similar to that of inositol 1,4,5-trisphosphate in releasing Ca^{2+} from the endoplasmic reticulum in permeabilized pancreatic-islet cells (Wolf *et al.*, 1986), and is able to mobilize cellular Ca^{2+} in rat islets of Langerhans (Metz *et al.*, 1987). In addition, we have shown that the administration of exogenous arachidonic acid to the perfused rat liver induces vasoconstriction,

Ca^{2+} -flux changes and glycogenolysis (Dieter *et al.*, 1987a,b).

Ca^{2+} -mobilizing agents such as phenylephrine, vasopressin, angiotensin, ADP, ATP and epidermal growth factor have been shown to interact synergistically with glucagon in stimulating Ca^{2+} uptake by the perfused rat liver (Altin & Bygrave, 1986, 1987). It therefore seemed important to test the possibility that in this system phosphatidic acid and arachidonic acid might similarly interact with glucagon (or cyclic AMP). We have examined this question in the present work, and report that phosphatidic acid and arachidonic acid each interacts synergistically with glucagon in stimulating Ca^{2+} influx by the perfused rat liver. In contrast with the influx of Ca^{2+} induced by glucagon and phosphatidic acid (and other Ca^{2+} -mobilizing agents), which is unaffected by indomethacin, that induced by the co-administration of glucagon and arachidonic acid is inhibited by this agent. This suggests that the mode of action of phosphatidic acid is similar to that of other Ca^{2+} -mobilizing hormones, but that the effects of arachidonic acid are mediated by products of cyclooxygenase.

METHODS AND MATERIALS

Male Wistar-strain rats (body wt. 280–350 g) fed *ad libitum* were used for all experiments. The rats were anaesthetized with sodium pentobarbitone (50 mg/kg) and the livers were perfused with Krebs–Henseleit (1932) bicarbonate medium, equilibrated with O_2/CO_2 (19:1), containing 1.3 mM- Ca^{2+} as described in Reinhart *et al.* (1982a). The perfusate Ca^{2+} and O_2 concentrations were monitored continuously with a Ca^{2+} -selective electrode

and a Clark-type oxygen electrode respectively, exactly as described previously (Reinhart *et al.*, 1982a; Altin & Bygrave, 1985). After a pre-perfusion period of 15–20 min, hormones and/or phosphatidic acid or arachidonic acid were administered at the specified concentrations by appropriate infusions with a pump-driven syringe for the period indicated in the Figures. Stock solutions of hormones were made up in Krebs–Henseleit medium, whereas phosphatidic acid and arachidonic acid were each made up in Krebs–Henseleit medium containing 10% (w/v) fatty acid-free bovine serum albumin and sonicated for 2–3 min at 37 °C. Indomethacin (20 μ M) was dissolved in dimethyl sulphoxide and then administered by addition to perfusion medium containing also 0.1% (w/v) bovine serum albumin. Under these conditions the presence of bovine serum albumin (final concn. < 0.03%) and dimethyl sulphoxide (final concn. < 0.02% v/v) did not induce any significant Ca^{2+} -flux change, nor alter significantly the responses induced by Ca^{2+} -mobilizing agents. For some experiments the samples of the effluent perfusate were collected for glucose determination by the glucose oxidase method as described by Reinhart *et al.* (1982b).

Chemicals and materials

Natural L- α -phosphatidic acid from egg yolk, synthetic L- α -phosphatidic acid (dipalmitoyl, dilauroyl and distearoyl forms), arachidonic acid, glucagon, phenylephrine, [Arg⁸]vasopressin, [Val⁵]angiotensin, dibutylcyclic AMP, indomethacin, bovine serum albumin and the glucose assay kit (510-A) were all obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. All other chemicals were of analytical grade.

RESULTS

Effect of phosphatidic acid administration on Ca^{2+} -flux changes, respiratory and glycogenolytic responses

Previous studies on the effect of phosphatidic acid on Ca^{2+} fluxes in isolated cells have provided conflicting results. For example, Barritt *et al.* (1981) reported that phosphatidic acid stimulates the net influx of Ca^{2+} in isolated hepatocytes, but more recently Moolenaar *et al.* (1986) have reported that phosphatidic acid stimulates Ca^{2+} efflux in human A341 cells. To our knowledge, no effect of phosphatidic acid on Ca^{2+} -flux movements in the perfused rat liver has yet been reported. As a prelude to studying possible synergistic actions of phosphatidic acid and glucagon, it therefore seemed relevant to examine first the effects of phosphatidic acid administered alone on Ca^{2+} -flux changes and other physiological responses in the perfused rat liver.

Figs. 1(a), 1(b) and 1(c) show respectively the Ca^{2+} , O_2 and glucose responses induced by administering 100 μ M-phosphatidic acid to the liver perfused with media containing either 1.3 mM- or 10 μ M- Ca^{2+} . At the higher extracellular Ca^{2+} concentration, phosphatidic acid induces a transient efflux of Ca^{2+} from the liver (120–150 nmol/g of liver). No net influx of Ca^{2+} can be detected until phosphatidic acid is removed. Moreover, Figs. 1(b) and 1(c) respectively, show that the phosphatidic acid-induced changes in O_2 uptake and glucose output remain stimulated for the time during which the compound is administered. It is noteworthy that phosphatidic acid induces Ca^{2+} efflux even when the

Fig. 1. Effect of phosphatidic acid administration on Ca^{2+} -flux changes, O_2 consumption and glucose output in the perfused rat liver

Livers were perfused with Krebs–Henseleit bicarbonate medium containing 1.3 mM- Ca^{2+} (—) or 10 μ M- Ca^{2+} (---; lowered from 1.3 mM to 10 μ M at 10 min of perfusion and thereafter for the remainder of the experiment) as detailed in the Methods and materials section. After a pre-perfusion period of 15 min, phosphatidic acid (PA; 100 μ M; bold horizontal line) was infused for 5 min as indicated by the arrows; 20 min later phenylephrine (Phe; 2 μ M, thin horizontal line) was also 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 is shown in (c), where each point is the result of a glucose determination carried out on a sample of the effluent perfusate collected at the indicated time. The traces are representative of those obtained from three separate experiments. The glucose data represent mean values; S.E.M. values were omitted for clarity, but ± 0.03 mM was never exceeded.

extracellular Ca^{2+} concentration is lowered to 10 μ M. In these experimental conditions the respiratory and glycogenolytic responses are transient (see Figs. 1b and 1c). These data suggest that the influx of extracellular Ca^{2+} is not required for triggering the induced respiratory and glycogenolytic effects, but that phosphatidic acid elicits these effects by releasing Ca^{2+} from intracellular stores.

The data in Fig. 2(a) show that the pre-administration of phenylephrine (5 μ M) results in a marked decrease in the Ca^{2+} efflux induced by phosphatidic acid. Also, the data in Fig. 2(b) show that the pre-administration of phosphatidic acid (150 μ M) markedly decreases the efflux of Ca^{2+} induced by phenylephrine. These results suggest that the Ca^{2+} release induced by phosphatidic acid originates from the same intracellular source as that

Fig. 2. Effect of sequential infusion of phosphatidic acid 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; $5 \mu\text{M}$; thin horizontal line) and then phosphatidic acid (PA; $150 \mu\text{M}$, bold line) were each infused for 5 min as indicated. The Ca^{2+} response induced is shown in (a). Fig. 2(b) shows the Ca^{2+} response obtained from a similar experiment in which the order of administration of phosphatidic acid and phenylephrine was reversed. Each trace is a representative of three experiments performed independently.

mobilized by phenylephrine, and hence that in this system phosphatidic acid elicits its effects by a mechanism which mimics that of other Ca^{2+} -mobilizing agents.

Specificity of phosphatidic acids in inducing physiological responses

Moolenaar *et al.* (1986) reported differences in the ability of different phosphatidic acids to induce a physiological response in human A431 carcinoma cells. We therefore decided to compare the effects of natural phosphatidic acid (from egg yolk) with those of different synthetic phosphatidic acids in the perfused rat liver. In our system, synthetic dipalmitoyl phosphatidic acid and synthetic dioleoyl phosphatidic acid elicited respiratory and glycogenolytic effects which were 30–50% (the Ca^{2+} flux changes induced were less than 10%) of those induced by a similar concentration of natural phosphatidic acid from egg yolk (results not shown). Also, synthetic dilauroyl phosphatidic acid elicited small effects only after it was removed whereas synthetic distearoyl phosphatidic acid elicited no detectable change in any of the responses studied. Such variation in the ability of these agents to elicit a response suggests that there is considerable specificity for the particular type of fatty acid chains in the phosphatidic acid molecule.

Fig. 3. Comparison of the Ca^{2+} -flux response induced by the co-administration of glucagon with phosphatidic acid, arachidonic acid or 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 (Phe; $2 \mu\text{M}$; —), phosphatidic acid (PA; $150 \mu\text{M}$; ----) or arachidonic acid (AA; $200 \mu\text{M}$; ····) was infused for the time indicated by the arrows. Similarly, the Ca^{2+} response obtained in separate experiments in which the infusion of phosphatidic acid, arachidonic acid and phenylephrine was preceded by a 4 min infusion of 10 nM -glucagon, which was then co-administered with each agent, is shown in (b). At this concentration the administration of glucagon induced only a small efflux of Ca^{2+} during the first 3–4 min, 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 $200 \mu\text{M}$ -cyclic AMP was administered instead of glucagon. Each trace is a representative of four or five experiments performed independently.

Ability of glucagon to potentiate phosphatidic acid- and arachidonic acid-induced Ca^{2+} influx

Data presented above (Figs. 1 and 2) establish that exogenous phosphatidic acid has properties similar to those of other Ca^{2+} -mobilizing hormones. Moreover, we have shown that the administration of arachidonic acid induces Ca^{2+} -flux changes and other physiological responses in the perfused rat liver (Dieter *et al.*, 1987a,b). Because glucagon has been found to interact synergistically with Ca^{2+} -mobilizing agents in stimulating Ca^{2+} influx in isolated hepatocytes (Morgan *et al.*, 1983; Mauger *et al.*, 1985; Poggioli *et al.*, 1986), and in the perfused liver (Altin & Bygrave, 1986, 1987), we conducted experiments to determine whether such

Fig. 4. Dose-response for the stimulation of Ca^{2+} influx by the co-administration of glucagon with phosphatidic acid or with arachidonic acid

Experiments were conducted exactly as described in the legend to Fig. 3(b), except that the concentrations of phosphatidic acid and arachidonic acid were varied. For each experiment the total net amount of Ca^{2+} influx, as represented by the area above the curve constituting the Ca^{2+} -influx response, was determined. Each point represents the net amount of Ca^{2+} influx obtained from separate experiments conducted at the indicated concentration of phosphatidic acid (PA; $\diamond$) and arachidonic acid (AA; $\bullet$). Each point is the mean $\pm$ S.E.M. of either three or four independent experiments conducted at the indicated concentration.

synergism could be observed also with glucagon and phosphatidic acid, or glucagon and arachidonic acid. Fig. 3(a) shows the Ca^{2+} -flux response induced by the administration of phosphatidic acid, arachidonic acid, and phenylephrine, when each was administered alone. By comparison, the effect of a 4 min pre-administration of 10 nM-glucagon is shown in Fig. 3(b). In each instance the administration of 10 nM-glucagon leads to a synergistic stimulation of Ca^{2+} uptake by the liver upon subsequent administration of 150 μM -phosphatidic acid, 200 μM -arachidonic acid or 2 μM -phenylephrine. Under these conditions, glucagon itself did not stimulate any net influx of Ca^{2+} when administered alone for the 9 min period (results not shown). The Ca^{2+} response induced by the co-administration of glucagon and phenylephrine was described previously (Altin & Bygrave, 1986) and is included here for comparison. The Ca^{2+} influx induced by the co-administration of glucagon and arachidonic acid was essentially complete after 5 min. However, the rate of Ca^{2+} influx induced by glucagon and phosphatidic acid (and also that induced by glucagon and phenylephrine) was approx. 60% of maximal at 5 min of co-

Table 1. Stimulation of Ca^{2+} influx by the co-administration of glucagon with phosphatidic acid, arachidonic acid 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 near-maximal concentrations of phosphatidic acid (150 μM), arachidonic acid (200 μM) or phenylephrine (2 μM). 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 $\pm$ S.E.M. of three or four independent experiments.

Additions	Ca^{2+} influx	
	Net amount (nmol/g of liver)	Maximum rate (nmol/min per g of liver)
Glucagon + phosphatidic acid	1170 $\pm$ 130	358 $\pm$ 35
Glucagon + arachidonic acid	484 $\pm$ 42	202 $\pm$ 24
Glucagon + phenylephrine	1050 $\pm$ 118	326 $\pm$ 38

administration, and the rate then decreased rapidly (within 1–2 min) when the stimuli were removed (Fig. 3). In each instance removal of the stimuli was subsequently followed by a slower efflux of Ca^{2+} over a period of 20–25 min. Although the magnitude of the Ca^{2+} influx and the minimal dose required to elicit the effect are different for each agent, the Ca^{2+} -flux response is qualitatively similar to that induced by glucagon and phenylephrine (Fig. 3), and glucagon and other Ca^{2+} -mobilizing agents such as vasopressin, angiotensin, ADP, ATP and epidermal growth factor (Altin & Bygrave, 1986, 1987).

The dose-response curves for the stimulation of Ca^{2+} uptake by glucagon and phosphatidic acid, and glucagon and arachidonic acid, are shown in Fig. 4. With 10 nM-glucagon, near-maximal stimulation of Ca^{2+} influx occurs at 150 μM -phosphatidic acid and 200 μM -arachidonic acid. The net amount of Ca^{2+} uptake, and the maximum rate of Ca^{2+} influx that is observed after the administration of these concentrations of phosphatidic acid and arachidonic acid, are given in Table 1 for comparison with phenylephrine. It is noteworthy that the Ca^{2+} response induced by the co-administration of glucagon and phosphatidic acid, or glucagon and phenylephrine, could be repeated by a second administration of these agents after a rest period of 20–25 min. However, the Ca^{2+} -influx response induced by glucagon and arachidonic acid was refractory, in that a second administration of the agents 20–30 min later failed to elicit a similar response (results not shown). Essentially identical results were obtained when 200 μM -cyclic AMP was used instead of 10 nM-glucagon (results not shown).

Effect of indomethacin on the Ca^{2+} response induced by the co-administration of glucagon and arachidonic acid, or glucagon and phosphatidic acid

Because it is known that arachidonic acid can be metabolized to eicosanoids, it seemed important to test

Fig. 5. Effect of indomethacin on the Ca^{2+} -influx response induced by the co-administration of glucagon with phosphatidic acid, with arachidonic acid, 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 continuously monitored with the Ca^{2+} -selective electrode as previously described. At 5 min of perfusion, and thereafter for the remainder of the experiment, the perfusion was carried out with media containing also $20 \text{ } \mu\text{M}$ -indomethacin (see the Methods and materials section). At 16 and 20 min of perfusion, glucagon (Glu; 10 nM ; broken horizontal lines) and phosphatidic acid (PA; $150 \text{ } \mu\text{M}$; bold horizontal line) were infused as indicated. Subsequently, at 40 min of perfusion glucagon (10 nM ; broken horizontal line), and 4 min later phenylephrine (Phe; $2 \text{ } \mu\text{M}$; thin horizontal line) were also administered as indicated. The Ca^{2+} response induced is shown in (a). The Ca^{2+} response obtained in a similar experiment in which arachidonic acid (AA) was administered instead of phosphatidic acid is shown in (b). Each trace is a representative of three independent experiments.

whether the cyclo-oxygenase inhibitor indomethacin could prevent the synergistic effects of glucagon and arachidonic acid (and perhaps glucagon and phosphatidic acid, or glucagon and phenylephrine) in stimulating Ca^{2+} uptake by the liver. Figs. 5(a) and 5(b) show that a 15 min pre-treatment with $20 \text{ } \mu\text{M}$ -indomethacin results in a complete inhibition of the Ca^{2+} response induced by the co-administration of glucagon and arachidonic acid. However, the Ca^{2+} influx response induced by the co-administration of glucagon and phosphatidic acid, and glucagon and phenylephrine, is not significantly affected by indomethacin (inhibition approx. 10%). These data suggest that cyclo-oxygenase products are the active metabolites in mediating the synergistic stimulation of Ca^{2+} influx by glucagon and arachidonic acid. The data

also indicate, by contrast, that such products are unlikely to be involved in the stimulation of Ca^{2+} influx that is induced by the co-administration of glucagon and phosphatidic acid, or glucagon and phenylephrine (see Fig. 5). Similarly, indomethacin failed to prevent Ca^{2+} influx induced by the synergistic action of glucagon with vasopressin and angiotensin instead of phenylephrine (results not shown).

DISCUSSION

This work has shown that the administration of exogenous phosphatidic acid induces Ca^{2+} efflux, and stimulates O_2 uptake and glucose output in the perfused rat liver. These effects, which are observed at both 1.3 mM and $10 \text{ } \mu\text{M}$ extracellular Ca^{2+} , are consistent with reports that phosphatidic acid induces glycogenolytic effects in isolated hepatocytes (Barritt *et al.*, 1981) and physiological responses in other cells (Salmon & Honeyman, 1980; Harris *et al.*, 1981; Ohsako & Deguchi, 1981). The results are not consistent, however, with reports that phosphatidic acid fails to stimulate glycogenolysis in cultured rat hepatocytes (Osugi *et al.*, 1984).

It has been reported that phosphatidic acid stimulates Ca^{2+} influx in cells in a Ca^{2+} -ionophoretic-like action (Putney *et al.*, 1980; Serhan *et al.*, 1981; Harris *et al.*, 1981; Ohsako & Deguchi, 1981). In the perfused liver, however, the net effect of phosphatidic acid is to induce Ca^{2+} efflux, most probably from the same intracellular stores as that released by the α -adrenergic agonist phenylephrine and other Ca^{2+} -mobilizing hormones (see Fig. 2). It is noteworthy also that Holmes & Yoss (1983) failed to observe Ca^{2+} influx induced by phosphatidic acid, albeit in phosphatidylcholine-containing membrane vesicles. Our result is also in agreement with the observation that at early times phosphatidic acid induces a loss of $^{45}\text{Ca}^{2+}$ from isolated hepatocytes pre-equilibrated with the radioisotope (Barritt *et al.*, 1981). Moreover, our data suggest that the action of phosphatidic acid with respect to mobilization of Ca^{2+} and the induction of respiratory and glycogenolytic effects in liver is similar to that of phenylephrine, an agent known to induce its effects by releasing Ca^{2+} through the breakdown of phosphoinositides and the generation of inositol 1,4,5-tris-phosphate (reviewed by Reinhart *et al.*, 1984; Berridge, 1984; Exton, 1985; Williamson *et al.*, 1985). The data presented thus give support to the findings by Moolenaar *et al.* (1986) that phosphatidic acid induces Ca^{2+} efflux and the breakdown of phosphoinositides in human A431 carcinoma cells.

It is not yet clear whether phosphatidic acid is acting through some receptors on the surface of the plasma membrane, or whether it enters the cell and activates phospholipase C directly, as proposed by Moolenaar *et al.* (1986). It is noteworthy, however, as also reported by Moolenaar *et al.* (1986), that natural phosphatidic acid from egg yolk is the most active of the phosphatidic acids tested.

The second major point arising from the present work concerns the ability of phosphatidic acid and of arachidonic acid each to interact synergistically with glucagon to stimulate Ca^{2+} influx. To our knowledge, no such action has yet been reported. The general pattern of the induced Ca^{2+} -influx response is similar to that evoked by the co-administration of glucagon and other Ca^{2+} -

mobilizing agents (cf. this work with Altin & Bygrave, 1986, 1987). However, the potency of phosphatidic acid differs from that of arachidonic acid in inducing Ca^{2+} influx when each is co-administered with glucagon (see Fig. 4), and the maximum amount of Ca^{2+} influx that is induced also differs between the two acids (Fig. 3 and Table 1).

Our finding that phosphatidic acid induces Ca^{2+} efflux even at low extracellular $[\text{Ca}^{2+}]$ (Figs. 1 and 2 of the present work), supports the observations that phosphatidic acid induces its effects by stimulating the breakdown of phosphoinositides (Moolenaar *et al.*, 1986). We therefore suggest that the synergistic action of glucagon and phosphatidic acid in stimulating Ca^{2+} influx need not involve phosphatidic acid itself, but that the Ca^{2+} influx is probably regulated by a mechanism similar to that which exists between cyclic AMP and other Ca^{2+} -mobilizing agents which are known to stimulate phosphoinositide metabolism. The particular aspect of phosphoinositide metabolism involved in regulating Ca^{2+} influx remains to be determined, although there is evidence for a possible involvement of inositol 1,3,4,5-tetrakisphosphate, a metabolite of inositol 1,4,5-trisphosphate (Irvine & Moor, 1986). We are mindful, on the other hand, that phosphatidic acid is rapidly produced as a consequence of the activation of receptors linked with the breakdown of phosphoinositides and mobilization of cellular Ca^{2+} (Berridge, 1984; Godfrey & Putney, 1984; Seyfred & Wells, 1984), and that the temporal accumulation of phosphatidic acid in the plasma membrane of hepatocytes stimulated with vasopressin (Seyfred & Wells, 1984) correlates well with the onset of Ca^{2+} influx that is observed in the presence of glucagon or cyclic AMP (see Altin & Bygrave, 1986).

A further and significant feature of the synergistic interaction of glucagon and arachidonic acid in stimulating Ca^{2+} influx is its sensitivity to the cyclo-oxygenase inhibitor indomethacin (Fig. 5). This suggests that arachidonic acid itself is not the active agent that interacts with glucagon (or cyclic AMP) to stimulate Ca^{2+} influx. Rather, it would seem that metabolites of cyclo-oxygenase activity are involved in mediating this effect. By contrast, under otherwise identical conditions the synergistic stimulation of Ca^{2+} influx induced by the co-administration of glucagon and phosphatidic acid, or glucagon and other Ca^{2+} -mobilizing agents, is essentially unaffected by indomethacin (see Fig. 5). This therefore suggests that products of cyclo-oxygenase are unlikely to be involved in eliciting the synergistic effects induced by these agents.

Because the liver parenchyma are thought not to be significant producers of prostanoids (Decker, 1985; Tran-Thi *et al.*, 1986), it would seem that the action of arachidonic acid in liver may involve the production of prostanoids by cell types other than hepatocytes. We envisage that such prostanoids, once released from these cells, could interact with hepatocytes through receptors that are themselves linked to the breakdown of phosphoinositides and a mobilization of cellular Ca^{2+} , in a manner that is similar to that induced by Ca^{2+} -mobilizing hormones (Creba *et al.*, 1983; Berridge, 1984). The synergism that exists between glucagon and arachidonic acid (Fig. 3) could then be explained by a mechanism analogous to that which exists between glucagon and the Ca^{2+} -mobilizing hormones. This notion is consistent with our proposal that the physiological responses

induced by arachidonic acid and zymosan (Dieter *et al.*, 1987a,b) and platelet-activating factor (Altin *et al.*, 1987) are mediated by prostanoids produced and released by different cell types within the liver.

Thus the present work has shown that both phosphatidic acid and arachidonic acid, metabolites that are produced as a consequence of the activation of a number of different receptors, themselves elicit Ca^{2+} -flux changes and physiological responses when administered exogenously to the perfused rat liver. Our data are consistent with the effects of arachidonic acid being mediated by products of cyclo-oxygenase, whereas those of phosphatidic acid appear to be induced by a mechanism similar to that of other Ca^{2+} -mobilizing agents. The particular product(s) of cyclo-oxygenase involved in mediating the effects of arachidonic acid, and the mechanism by which phosphatidic acid seemingly stimulates phosphoinositide breakdown, remain to be determined.

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