Platelet-activating factor stimulates metabolism of phosphoinositides via phospholipase A_2 in primary cultured rat hepatocytes

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Abstract Addition of platelet-activating factor (PAF) to cells doubly labeled with [^{14}C]glycerol plus [^{3}H]arachidonic acid resulted in a transient decrease of [^{14}C]glycerol-labeled phosphatidylinositol (PI) and a transient increase of [^{14}C]glycerol-labeled lysophosphatidylinositol (LPI). [^{3}H]Arachidonate-labeled PI, on the other hand, decreased in a time-dependent manner. The radioactivity in phosphatidylethanolamine, phosphatidylcholine, sphingomyelin, and phosphatidylserine did not change significantly. The ^{3}H/^{14}C ratio decreased in PI in a time-dependent manner, suggesting the involvement of a phospholipase A_2 activity. Although PAF also induced a gradual increase of diacylglycerol (DG), the increase of [^{14}C]glycerol-labeled DG paralleled the loss of triacyl [^{14}C]glycerol and the ^{3}H/^{14}C ratio of DG was 16 times smaller than that of PI. Thus, DG seemed not to be derived from PI. In myo- [^{3}H]inositol-prelabeled cells, PAF induced a transient decrease of [^{3}H]phosphatidylinositol-4,5-bis-phosphate (TPI) and [^{3}H]phosphatidlyinositol-4-phosphate (DPI) at 1 min. PAF stimulation of cultured hepatocytes prelabeled with $^{32}P_i$ induced a transient decrease of [^{32}P]polyphosphoinositides at 20 sec to 1 min. [^{32}P]LPI appeared within 10 sec after stimulation and paralleled the loss of [^{32}P]PI. [^{3}H]Inositol triphosphate, [^{3}H]inositol diphosphate, and [^{3}H]inositol phosphate, which increased in a time-dependent manner upon stimulation with adrenaline, did not accumulate with the stimulation due to PAF. These observations indicate that PAF causes degradation of inositol phospholipids via phospholipase A_2 and induces a subsequent resynthesis of these phospholipids.—**Okayasu, T., K. Hasegawa, and T. Ishibashi.** Platelet-activating factor stimulates metabolism of phosphoinositides via phospholipase A_2 in primary cultured rat hepatocytes. *J. Lipid Res.* 1987. **28:** 760–767.

Supplementary key words phosphatidylinositol • polyphosphatidylinositides • inositol polyphosphates • phospholipase C

Platelet-activating factor (PAF), identified as 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (1, 2), is a biologically active phospholipid that has potent effects on inducing the aggregation of platelets (3–5) and the secretion of granular material from neutrophils (6, 7). The biological mechanisms by which PAF elicit physiological responses are not fully understood. In platelets, PAF has been shown to stimulate inositol phospholipid turnover (8–11), the accumulation of phosphatidic acid (PA) or diacylglycerol (DG) (8–12), and the release of arachidonic acid (11–13) and inositol phosphates (14). These biological responses are thought to be related to the release of Ca^{2+} from a cellular pool and it has been proposed that inositol-1,4,5-triphosphate (IP_3), a breakdown product of phosphatidylinositol-4,5-biphosphates (TPI), may act as a second messenger (15). Similar responses stimulated by PAF are reported in macrophages (16), rat renal mesangial cells (17), and neutrophils (18, 19). Blank et al. (20) measured PAF metabolism in vivo and found that the liver contained one of the highest levels of PAF and its metabolites. Infusion of PAF into perfused rat liver causes a rapid stimulation of hepatic glycogenolysis (21). It is known that vasopressin, angiotensin, and adrenaline stimulate polyphosphoinositide turnover and evoke an activation of glycogen phosphorylase in the liver (22–24). Addition of PAF to ^{32}P-labeled isolated rat hepatocytes resulted in rapid decreases of [^{32}P]TPI and [^{32}P]phosphatidylinositol-4-phosphate (DPI) but did not activate glycogen phosphorylase (21, 25). Charest et al. (26) recently reported that vasopressin, angiotensin, and adrenaline stimulated IP_3 formation and activated glycogen phosphorylase in hepatocytes, but no increase of IP_3 was observed in cells stimulated with PAF (26). To explore the biological mechanism by which PAF causes physiological responses in the liver, we examined the metabolic behavior of exogeneous PAF in cultured hepatocytes and found a new metabolic pathway which is different from that of platelets and neutrophils (27). In the present study we examined the effects of PAF on the metabolism of phospholipids in primary cultured adult rat hepatocytes prelabeled with [^{14}C]glycerol

Abbreviations: PAF, platelet-activating factor; PA, phosphatidic acid; DG, diacylglycerol; IP_3, inositol triphosphate; TPI, phosphatidylinositol-4,5-bisphosphate; LPI, lysophosphatidylinositol; LPC, lysophosphatidylcholine; PI, phosphatidylinositol; DPI, phosphatidylinositol-4-phosphate; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TG, triacylglycerol; IP, inositol monophosphate; IP_2, inositol diphosphate; FFA, free fatty acids; TLC, thin-layer chromatography.

plus [^{3}H]arachidonic acid or myo[^{3}H]inositol or $^{32}P_i$. We found that inositol phospholipids were selectively hydrolyzed by phospholipase A_2 and subsequently resynthesized.

MATERIALS AND METHODS

Materials

[5,6,8,9,11,12,14,15-^{3}H(N)]Arachidonic acid (87.2 Ci/mmol), $^{32}P_i$(carrier-free), [^{14}C(U)]glycerol (8.7 mCi/mmol) and myo[2-^{3}H(N)]inositol (16.5 Ci/mmol) were purchased from New England Nuclear. PAF was a generous gift from Dr. Toshio Muramatsu. Yohimbine and propranolol were obtained from Wako Chemical Industries; (−)-adrenaline bitartrate, collagenase (type IV), TPI, and DPI were from Sigma; and Dowex 1-X8 ion exchange resin, 200–400 mesh was from Bio-Rad. All other chemicals and solvents used were of the highest analytical grade available.

Primary culture of adult rat hepatocytes and labeling of cells

Hepatocytes were isolated by collagenase perfusion from livers of 150–200 g male Wistar rats fed a standard laboratory chow as described previously (28). Isolated hepatocytes were plated in 21-cm^2 tissue culture dishes (Falcon Labware) at a density of 0.8 $\times 10^5$/cm^2 in William's medium E (Flow Laboratories, Inc.) containing 10% heat-inactivated fetal calf serum (Flow Laboratories, Inc.), 10 μg/ml streptomycin, 10 IU/ml penicillin and 0.02 U/ml insulin. After incubation at 37°C in a humidified atmosphere of 5% CO_2/95% air for 60–90 min to allow the cells to attach, the cultured cells were rinsed and incubated in a medium containing either 6 μCi/ml of [^{14}C]glycerol or 6 μCi/ml of myo[^{3}H]inositol or 6 μCi/ml of $^{32}P_i$. Cells were incubated for another 24 hr in this medium. For the double-labeling of hepatocytes, after the first 24-hr incubation with [^{14}C]glycerol, 0.6 μCi/ml of [^{3}H]arachidonic acid was added to the medium and cells were incubated for another 24 hr. Separate experiments showed that these prelabeling periods yielded maximal incorporation of the radioactive precursors into the major phospholipids and neutral lipids. After preincubation, cells were rinsed three times and placed in isotope-free simple William's medium and incubated for 1 hr before addition of PAF or hormones. For analysis of myo[^{3}H]inositol-prelabeled cells, 10 mM LiCl was added to the simple William's medium.

Analytical procedures

When cellular lipids were obtained from doubly prelabeled cells or $^{32}P_i$-prelabeled cells, each incubation was conducted in triplicate for the desired length of time in the presence of 10 nM PAF, and stopped by the addition of 1.25 vol of methanol–12 M HCl 80:1 (by vol). Lipids were extracted as described previously (27) using the procedure of Bligh and Dyer (29). The radioactivity of aliquots of the extracted lipids was counted by using an Aloka liquid scintillation counter (LSC-900). The rest of the lipids were separated on layers of silica gel plate (Merck) using the following solvent systems: I, chloroform–methanol–20% methylamine 60:36:10 (by vol) (30) for separation of phospholipids; II, hexane–diethyl ether–acetic acid 60:40:1 (by vol) for separation of neutral lipids; III, chloroform–pyridine–formic acid 50:35:5 (by vol) (8) for separation of phosphatidic acid (PA). For a good separation of lysophosphatidylinositol (LPI), lysophosphatidylcholine (LPC), and PA from other phospholipids, two-dimensional chromatography using chloroform–methanol–acetic acid–water 75:45:12:3 (by vol) in the first dimension and chloroform–methanol–concentrated ammonia–water 70:30:0.5:4 (by vol) in the second dimension was performed (31). Separated lipids were located by iodine vapor and identified by comparison with authentic standards. Once located, each area was scraped into a vial and the radioactivity was determined. The data were calculated from the percentage radioactivity of each fraction in total radioactivity recovered on the TLC plate and the radioactivity of extracted total lipids.

When myo[^{3}H]inositol-prelabeled hepatocytes were treated with PAF or 30 μM adrenaline plus 1 μM propranolol plus 1 μM yohimbine, incubations were terminated by rapid aspiration of the incubation medium, and 1 ml of cold 10% (w/v) trichloroacetic acid was added. Cells were scraped and precipitate was collected by centrifugation (500 *g* for 15 min). The precipitate was washed once with 1 ml of 5% (w/v) trichloroacetic acid and once with 1 ml of water, both of which were first used to rinse the dish before washing. After the removal of trichloroacetic acid with five diethylether extractions and neutralization with 6.25 mM sodium tetraborate, the trichloroacetic acid-soluble [^{3}H]inositol phosphates were separated from each other with 1 ml of Dowex-1 (formate form) using the method of Berridge et al. (32). A 5-ml portion of each fraction was mixed with 10 ml of ACSII (Amersham) and radioactivity was counted with an Aloka liquid scintillation counter. The [^{3}H]inositol lipids in the trichloroacetic acid precipitate were extracted by sequential treatment to get the largest yield of TPI and DPI: twice with 1.5 ml of chloroform–methanol–12 M HCl 100:100:1 (by vol) and once with 1 ml of chloroform–methanol–12 M HCl 200:100:1 (by vol) as described by Creba et al. (22). The extracts were separated by thin-layer chromatography using solvent system IV, chloroform–methanol–aq. 4 M NH_3 9:7:2 (by vol) (33). Spots were located and scraped off, and calculations were as described above.

RESULTS

PAF-induced changes in cells prelabeled with [^{14}C]glycerol plus [^{3}H]arachidonic acid

The metabolic changes of lipids in primary cultured adult rat hepatocytes upon the addition of PAF were examined in cells prelabeled with [^{14}C]glycerol (for 48 hr) plus [^{3}H]ara-

chidonic acid (for 24 hr). Under these conditions, cultured hepatocytes incorporated about 1.0–1.7% and 30–39% of [^{14}C]glycerol and [^{3}H]arachidonic acid added, respectively, into PC (23.1 ± 0.7% of total ^{14}C radioactivity incorporated, 50.0 ± 1.4% of total ^{3}H radioactivity incorporated), PE (5.02 ± 0.14% of ^{14}C, 20.4 ± 0.7% of ^{3}H), PI (3.3 ± 0.3% of ^{14}C, 9.9 ± 0.9% of ^{3}H), TG (55.4 ± 1.2% of ^{14}C, 10.2 ± 0.2% of ^{3}H), DG (10.3 ± 0.4% of ^{14}C, 2.3 ± 0.1% of ^{3}H), free fatty acids (0.7 ± 0.1% of ^{3}H), LPC (0.6 ± 0.04% of ^{14}C), LPI (0.61 ± 0.06% of ^{14}C) and PA (0.21 ± 0.04% of ^{14}C, 0.54 ± 0.05% of ^{3}H).

Addition of 10 nM PAF resulted in a transient decrease of [^{14}C]glycerol-labeled PI. [^{14}C]Glycerol-labeled PI decreased to a minimum at 5 min, which amounted to 20% of the control value, and gradually increased thereafter and reached to the initial level at 60 min. [^{14}C]glycerol-labeled LPI increased gradually reaching its maximum at 5 min and gradually decreased thereafter. On the other hand, [^{3}H]arachidonate-labeled PI decreased in a time-dependent manner up to 60 min when stimulated with PAF (**Fig. 1**) and [^{3}H]arachidonate-labeled LPI was scarcely detected. The radioactivity of PE and PC did not decline (Fig. 1). The radioactivity of phosphatidylserine, sphingomyelin, and LPC did not change significantly (data not shown). The [^{3}H]arachidonate/[^{14}C]glycerol ratio decreased significantly in a time-dependent manner in PI after the addition of PAF (**Fig. 2**), suggesting the involvement of phospholipase A_2 activity in PI metabolism. When 10 nM PAF was added, the radioactivity of [^{3}H]arachidonate-labeled free fatty acids (FFA) increased (**Fig. 3**). A 3.5-fold increase was evident within 20 sec and the content of [^{3}H]arachidonate-labeled FFA stayed at a 3- to 2-fold increased level up to 30 min. PAF also induced a gradual increase of [^{3}H]arachidonate-labeled DG in doubly labeled cells (Fig. 1). Since the increase of [^{14}C]glycerol-labeled DG paralleled the decrease of [^{14}C]glycerol-labeled TG, and the [^{3}H]arachidonate/[^{14}C]glycerol ratio of DG was 16 times smaller than that of PI (0.95 ± 0.11, 15.9 ± 0.65, respectively), it seemed that DG was not derived from PI but from TG or other lipids that had a low [^{3}H]arachidonate/[^{14}C]glycerol ratio. Also, upon stimulation with PAF, [^{3}H]arachidonate-labeled PA increased in a time-dependent manner

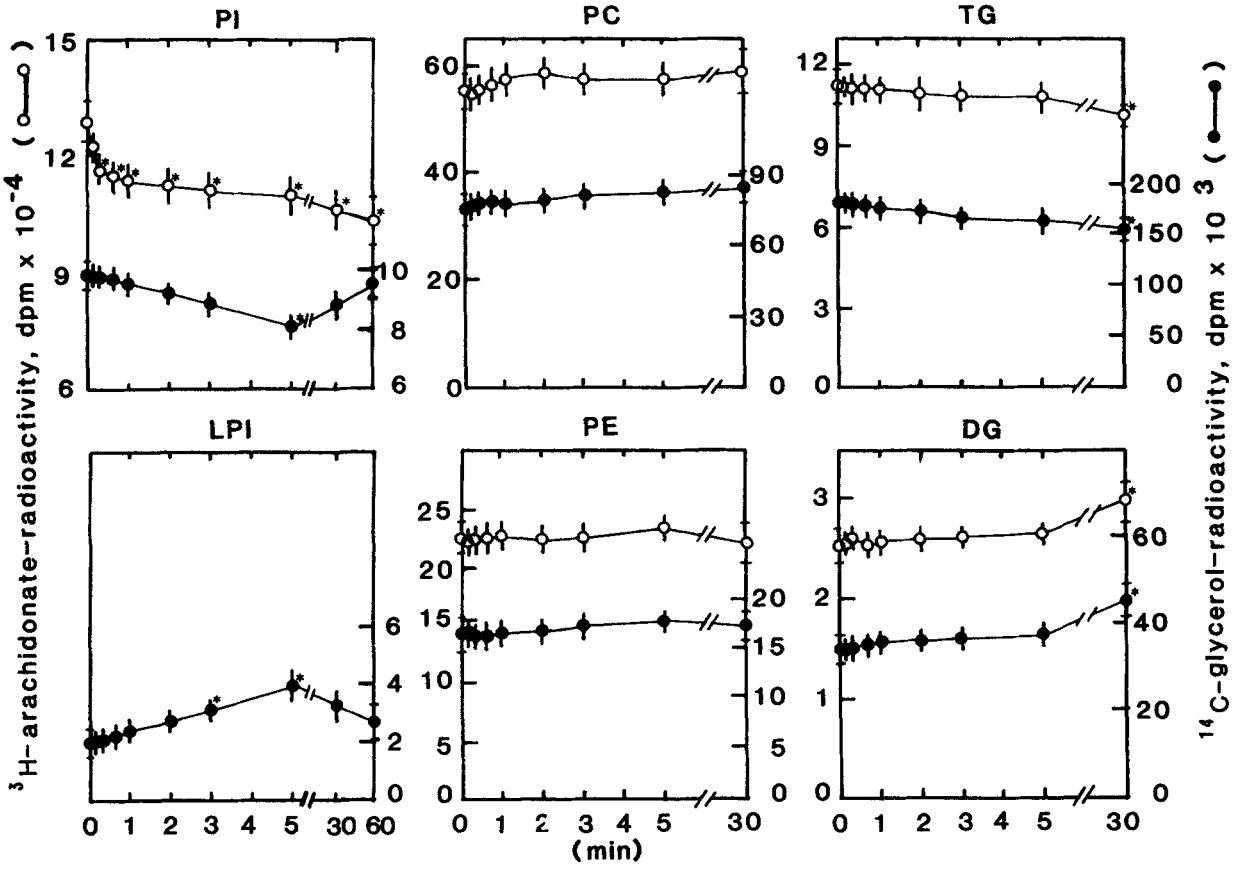

Fig. 1. Time course of PAF-induced changes of lipids in cultured hepatocytes. Primary cultures of rat hepatocytes were doubly labeled with [^{3}H]arachidonic acid plus [^{14}C]glycerol as described in Materials and Methods before the addition of 10 nM PAF. Incubations were terminated at the indicated times and the radioactivity present in each lipid was determined. Results are mean ± SD for three separate hepatocyte preparations expressed as dpm/1.7 × 10^{6} cells; (*), significantly different from 0 time ($P < 0.01$); (○), [^{3}H]arachidonate-radioactivity; (●), [^{14}C]glycerol-radioactivity.

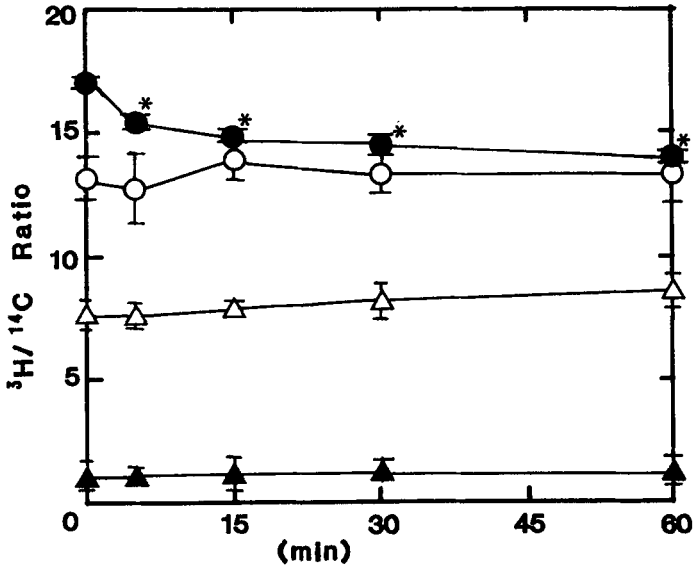

Fig. 2. Time course of PAF-induced changes in the [^{3}H]arachidonate/[^{14}C]glycerol ratio of lipids in cultured hepatocytes prelabeled with [^{3}H]arachidonic acid plus [^{14}C]glycerol. Hepatocytes were prepared and incubated with PAF and analyzed as described in the legend to Fig. 1. The $^3H/^{14}C$ ratio (dpm/dpm) was determined in PI (●), PE (○), PC (△) and DG (▲).

up to 2 min and then decreased to 1.2-fold at 5 min (Fig. 3). Its [^{3}H]arachidonate/[^{14}C]glycerol ratio was 3.0–4.0, which was 4 to 5 times smaller than that of PI. The amount of [^{3}H]arachidonate acid released was comparable to the loss of [^{3}H]arachidonate-labeled PI. These results indicate that PI is degraded mainly by phospholipase A_2 in PAF-stimulated cultured rat hepatocytes.

PAF-induced changes of inositol phospholipids in cells prelabeled with myo[^{3}H]inositol

The time course and magnitude of hepatic polyphosphoinositide changes induced by PAF were investigated in primary cultured adult rat hepatocytes prelabeled with myo[^{3}H]inositol for 24 hr. The incorporation of the radioactive precursor into the hepatocytes was about 1.8% and the distribution of labeling was as follows: PI (67.3 ± 3.0%), LPI (5.2 ± 0.3%), DPI (1.3 ± 0.1%), TPI (1.8 ± 0.2%), inositol (22.2 ± 3%), glycerophosphoinositol (0.73 ± 0.07%), IP (1.11 ± 0.10), IP_2 (0.1 ± 0.01%), IP_3 (0.07 ± 0.008%). Ten nM PAF induced a transient decrease of [^{3}H]inositol-labeled TPI and [^{3}H]inositol-labeled DPI to about 72% and 84% of time 0 at 1 min, respectively, and then labeled TPI and DPI returned toward their initial levels at 5 min. Further incubation resulted in an increase of labeled TPI and DPI over their initial levels (**Fig. 4**). [^{3}H]Inositol-labeled PI did not decline but rather increased gradually upon stimulation by PAF. [^{3}H]Inositol-labeled LPI increased significantly after 5 min. **Fig. 5** shows the dose–response curve of the PAF-stimulated depletion of TPI and DPI. These data represent measurements taken during 1-min periods. In both polyphosphoinositides, 10 nM PAF caused maximal depletion. Without PAF stimulation there was no significant change.

[^{3}H]Inositol-labeled IP_2 and [^{3}H]inositol-labeled IP_3, which increased in a time-dependent manner upon stimulation with 30 μM adrenaline plus 1 μM yohimbine and 1 μM propranolol, did not accumulate in the hepatocytes stimulated with 10 nM PAF (**Fig. 6**). The stimulation of hepatocytes with PAF did not cause an accumulation of [^{3}H]inositol-labeled IP. These observations indicate that PAF degrades polyphosphoinositide via phospholipase A_2 and induces an uptake of myo[^{3}H]inositol into inositol phospholipids.

PAF-induced changes in primary cultured adult rat hepatocytes prelabeled with $^{32}P_i$

The time course of hepatic phosphoinositides and PA changes induced by PAF were investigated in ^{32}P-prelabeled cells (**Fig. 7**). The stimulation of hepatocytes with PAF for 5 min induced degradation of about 10% of [^{32}P]PI with the concomitant formation of [^{32}P]LPI plus [^{32}P]PA. [^{32}P]LPI appeared within 10 sec and increased significantly in a time-dependent manner. [^{32}P]LPI accounted for 80% of the total amount of the degraded [^{32}P]PI at 5 min. [^{32}P]PA, on the other hand, did not increase significantly. This precursor–product relationship of PI and LPI strongly suggested the involvement of phospholipase A_2 in PI degradation caused by PAF. PAF induced a transient decrease of [^{32}P]TPI to 70% at 20 sec and of [^{32}P]DPI to 77% at

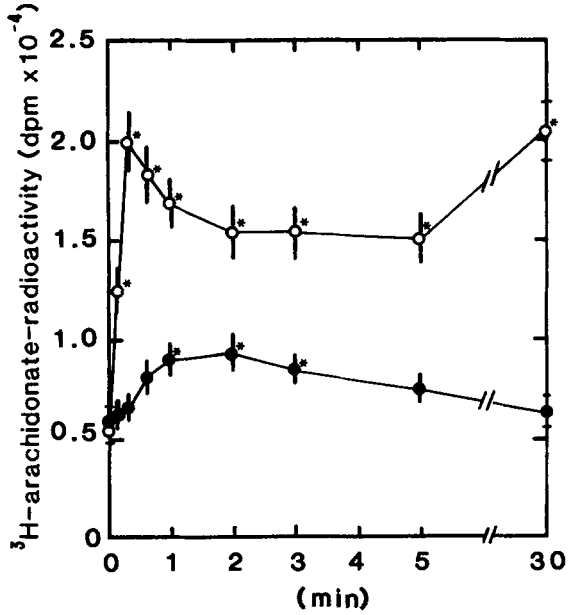

Fig. 3. Time course of the PAF-induced changes of free fatty acid and phosphatidic acid in cultured hepatocytes. Cells were doubly labeled with [^{3}H]arachidonic acid and [^{14}C]glycerol and exposed to 10 nM PAF for the indicated times. The ^{3}H content of FFA (○) and PA (●) was determined and expressed as described in the legend to Fig. 1.

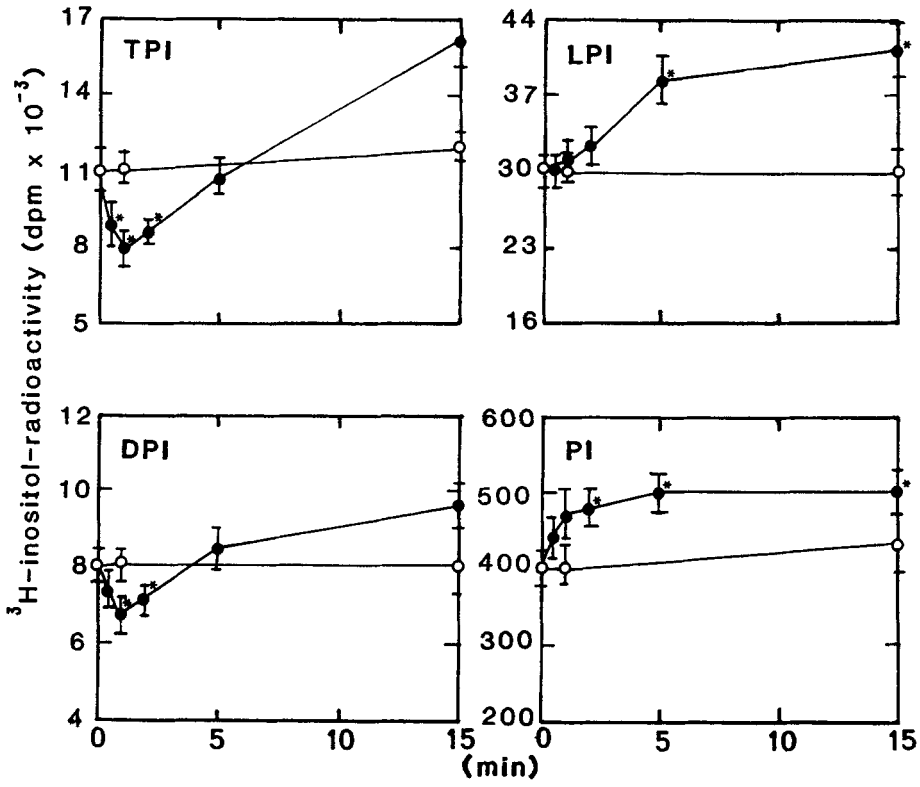

Fig. 4. Time course of the changes in inositol phospholipids induced by PAF. Primary cultures of rat hepatocytes were prelabeled with myo[^{3}H]inositol as described in Materials and Methods. Cells were rinsed and placed in isotope-free William's medium containing 10 mM LiCl for 1 hr before addition of 10 nM PAF. Incubation was terminated at the indicated times and the radioactivity of TPI, DPI, LPI, and PI was determined as described in Materials and Methods. Data are expressed as described in the legend to Fig. 1; (○), control; (●), 10 nM PAF.

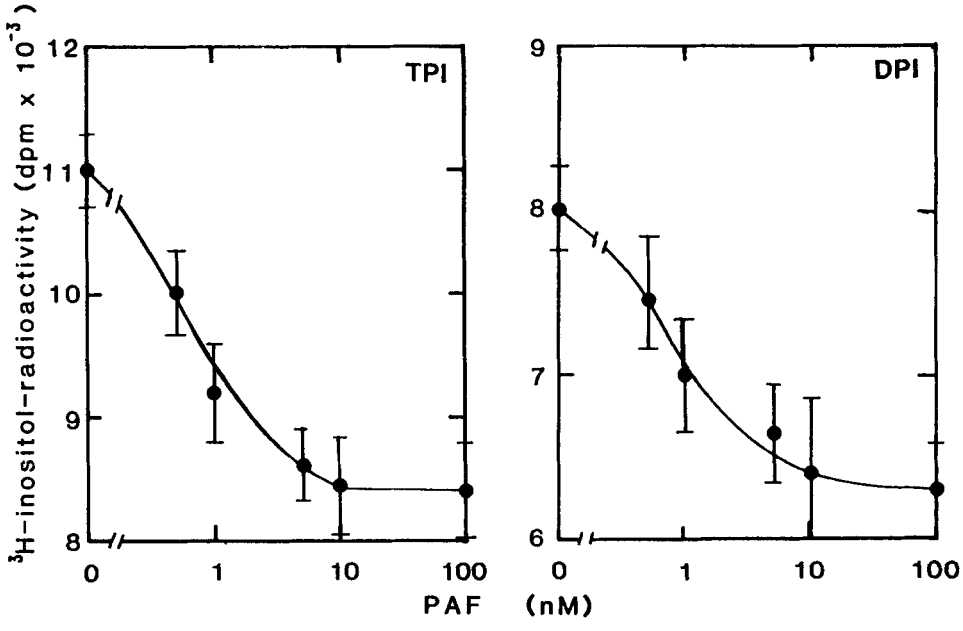

Fig. 5. Dose-dependent effect of PAF on TPI and DPI depletion at 1 min. Primary cultures of rat hepatocytes were prelabeled with myo[^{3}H]inositol and treated as described in the legend to Fig. 4. Cells were exposed to the indicated concentrations of PAF for 1 min. Radioactivity of TPI and DPI is expressed as described in the legend to Fig. 4.

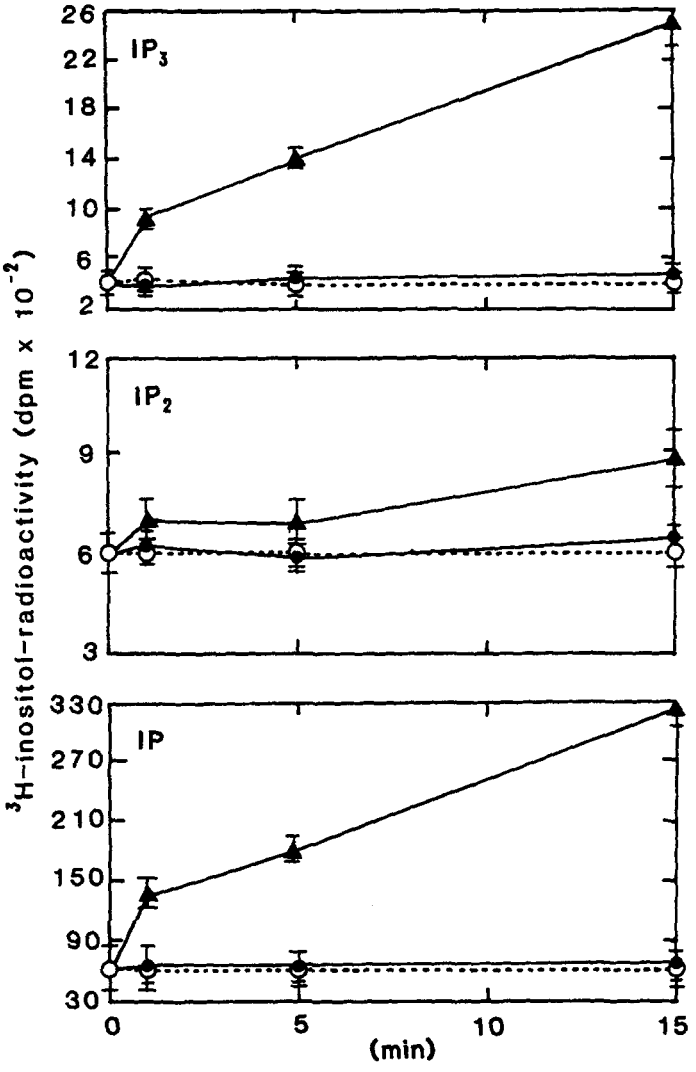

Fig. 6. Time course of the changes in IP, IP_2, and IP_3 induced by adrenaline and PAF. Primary cultured rat hepatocytes were prelabeled with myo[^{3}H]inositol and treated as described in the legend to Fig. 4. Cells were exposed to 10 nM PAF (●) or 30 μM adrenaline plus 1 μM yohimbine and 1 μM propranolol (▲) for the indicated times and the ^{3}H content of IP_3, IP_2, and IP was determined; (○), control.

1 min and then labeled TPI and DPI returned toward their initial levels at 5 min. No significant change was observed in the absence of PAF during 5 min incubation.

DISCUSSION

PAF is a potent lipid mediator strongly implicated in the pathogenesis of acute allergic and inflammatory reaction. In platelets, PAF has been shown to stimulate the PI cycle through a rapid degradation of PI by a specific phospholipase C. The resultant DG is converted, first to PA and then to CDP-diacylglycerol and PI (8, 11). PAF has also been shown to stimulate the release of arachidonic acid and its metabolites from membrane phospholipids in platelets (11–13). PAF causes a rapid but transient breakdown in TPI and DPI as well (10), and it has been suggested that PAF-induced formation of inositol polyphosphate is an early response specific to PAF and may play a role in the initiation of platelet activation by Ca^{2+} mobilization (14). However, little information is available regarding the effects of PAF on cells other than platelets.

In our experiments, the addition of PAF caused a rapid hydrolysis of PI and an increase of LPI in primary cultured rat hepatocytes prelabeled with [^{14}C]glycerol plus [^{3}H]arachidonic acid. The radioactivity of PC, PE, and other major phospholipids did not decrease upon the addition of PAF up to 100 μM which caused 20% cell death, measured by the release of lactate dehydrogenase (data not shown). The [^{3}H]arachidonate/[^{14}C]glycerol ratio decreased significantly in PI and [^{3}H]arachidonic acid appeared within 10 sec and increased 3.5-fold upon stimulation with PAF. PAF also increased [^{3}H]inositol-labeled LPI in myo[^{3}H]inositol-labeled cultured hepatocytes without an accumulation of [^{3}H]inositol-labeled IP. One might suggest that separate pools of PI were labeled with [^{3}H]arachidonate, [^{14}C]glycerol, and [^{3}H]inositol and responded differently following stimulation. However, since we labeled cells for 24 or 48 hours, separate pools of PI such as proposed by Monaco (34) were not the case in our system presented here. The precursor-product relationship of PI and LPI was clearly demonstrated in $^{32}P_i$-labeled hepatocytes after the addition of PAF (Fig. 7). These data suggest that PAF metabolizes PI mainly by phospholipase A_2. Degradation of PI by phospholipase A_2 was reported in platelets (8), aortic smooth muscle cells, and renal epithelial cells (35, 36). The activation of PI-specific phospholipase C is also found in these cells. In cultured hepatocytes, however, the activation of PI-specific phospholipase C was not evident, since [^{3}H]inositol-labeled IP did not accumulate for up to 15 min of incubation time after the addition of PAF in the presence of 10 mM LiCl. The [^{3}H]arachidonate/[^{14}C]glycerol ratios of DG and PA were much lower than that of PI. An increased acylation of PI is suggested since the decrease of [^{14}C]glycerol-labeled PI was transient while the decrease of [^{3}H]arachidonate-labeled PI was time-dependent in doubly prelabeled hepatocytes, and the ratio of labeled arachidonic acid to unlabeled arachidonic acid in total lipid was calculated to be less than 0.008. Increased deacylation promoted by PAF is also suggested in neutrophils (19).

There is a common belief held by many that the agonist-induced breakdown of PI is due to phosphorylation to form polyphosphoinositide rather than a direct action of phospholipase C on PI. In our experiment, PAF induced a transient decrease of [^{3}H]inositol-labeled TPI and [^{3}H]inositol-labeled DPI in cultured hepatocytes and further incubation resulted in an increase over their initial levels. It is noteworthy that in hepatocytes [^{14}C]glycerol-labeled LPI or [^{32}P]LPI increased concomitantly with the decrease of [^{14}C]glycerol-labeled PI or [^{32}P]PI, respectively. The pro-

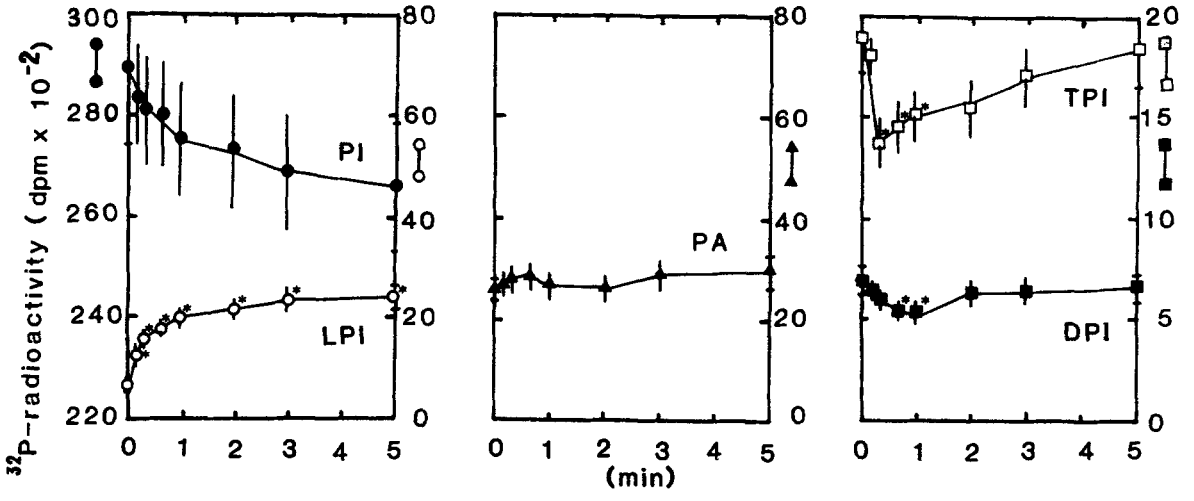

Fig. 7. Time course of the changes in inositol phospholipids and phosphatidic acid induced by PAF in cultured hepatocytes prelabeled with $^{32}P_i$. Cells were prelabeled with $^{32}P_i$ for 24 hr and incubated with 10 nM PAF for the indicated times. The radioactivitiy of PI, LPI, PA, TPI, and DPI was determined as described in Materials and Methods. Results are expressed as described in the legend to Fig. 1.

posed degradation products of polyphosphoinositide by phospholipase C, IP_3, and IP_2 did not accumulate, so this transient decrease could be due to activation of phospholipase A_2. This concurs with the finding of Charest et al. (26). Labeled TPI and DPI also decreased upon stimulation with PAF in [^{3}H]arachidonic acid-labeled hepatocytes (data not shown). The increase of [^{3}H]inositol-labeled PI could be the result of stimulated incorporation of internal myo[^{3}H]inositol since labeled hepatocytes contained free myo[^{3}H]inositol even after three washings. PAF-induced incorporation of myo[^{3}H]inositol into inositol phospholipids suggests that PAF stimulates the resynthesis of inositol phospholipids.

The effects of PAF on noninflammatory tissues such as the liver are not clearly understood. Infusion of PAF into perfused rat liver caused a rapid stimulation of hepatic glycogenolysis (21) but addition of PAF to isolated rat hepatocytes did not result in an activation of glycogen phosphorylase, though a rapid breakdown of TPI and DPI was observed (21, 25). Buxton et al. (37) has reported that glycogenolysis in the perfused liver in response to PAF may be a result of the hemo-dynamic effects of PAF rather than the direct effect on hepatocytes. In the present study we have shown that PAF stimulates the degradation and resynthesis of inositol phospholipids in primary cultured rat hepatocytes through a mechanism involving the activation of phospholipase A_2 rather than via the PI cycle or polyphosphoinositide turnover mechanisms.

Gray and Strickland (38) have demonstrated the presence of a phospholipase A_2 in bovine or rat brain that is specific for PI and proposed that this might function in a deacylation-reacylation cycle for modifying the fatty acid distribution in PI. We have demonstrated here the presence of phospholipase A_2 in rat liver which shows a specificity for phosphoinositides and releases arachidonic acid upon stimulation with PAF. Studies are in progress in our laboratory to shed more light on the physiological significance of this PI-specific phospholipase A_2 and the effect of PAF and released arachidonic acid on the liver.

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REFERENCES

1. Benveniste, J., M. Tencé, P. Varenne, J. Bidault, C. Boullet, and J. Polonsky. 1979. Semi-synthèse et structure proposée du facteur activant les plaquettes (P.A.F.): PAF-acether, un alkyl ether analogue de la lysophosphatidylcholine. C. R. *Acad. Sci. (D) Paris.* **289:** 1037-1040.
2. Hanahan, D. J., C. A. Demopoulos, J. Liehr, and R. N. Pinckard. 1980. Identification of platelet-activating factor isolated from rabbit basophils as acetyl glycerylether phosphorylcholine. *J. Biol. Chem.* **255:** 5514-5516.
3. Benveniste, J., C. G. Cochrane, and P. M. Henson. 1972. Leukocyte-dependent histamine release from rabbit platelets: the role of IgE, basophils and a platelet-activating factor. *J. Exp. Med.* **136:** 1356-1377.
4. Henson, P. M. 1977. Activation of rabbit platelets by platelet-activating factor derived from IgE-sensitized basophils. *J. Clin. Invest.* **60:** 481-490.
5. McManus, L. M., D. J. Hanahan, and R. N. Pinckard. 1981. Human platelet stimulation by acetyl glyceryl ether phosphorylcholine. *J. Clin. Invest.* **67:** 903-906.
6. O'Flaherty, J. T., R. L. Wykle, C. H. Miller, J. C. Lewis, M. Waite, D. A. Bass, C. E. McCall, and L. R. DeChatelet. 1981. 1-O-Alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholines, a novel class of neutrophil stimulants. *Am. J. Pathol.* **103:** 70-78.
7. Shaw, J. O., R. N. Pinckard, K. S. Ferrigni, L. M. McManus, and D. J. Hanahan. 1981. Activation of human neutrophils with 1-O-hexadecyl/octadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine (platelet activating factor). *J. Immunol.* **127:** 1250-1255.

8. Shukla, S. D., and D. J. Hanahan. 1982. AGEPC (platelet activating factor) induced stimulation of rabbit platelets: effects on phosphatidylinositol, di- and triphosphoinositides and phosphatidic acid metabolism. *Biochem. Biophys. Res. Commun.* **106:** 697–703.
9. Ieyasu, H., Y. Takai, K. Kaibuchi, M. Sawamura, and Y. Nishizuka. 1982. A role of calcium-activated, phospholipid-dependent protein kinase in platelet-activating factor-induced serotonin release from rabbit platelets. *Biochem. Biophys. Res. Commun.* **108:** 1701–1708.
10. Billah, M. M., and E. G. Lapetina. 1983. Platelet-activating factor stimulates metabolism of phosphoinositides in horse platelets: possible relationship to Ca^{2+} mobilization during stimulation. *Proc. Natl. Acad. Sci. USA.* **80:** 965–968.
11. Lapetina, E. G. 1982. Platelet-activating factor stimulates the phosphatidylinositol cycle: appearance of phosphatidic acid is associated with the release of serotonin in horse platelets. *J. Biol. Chem.* **257:** 7314–7317.
12. Siess, W., P. C. Weber, and E. G. Lapetina. 1984. Activation of phospholipase C is dissociated from arachidonate metabolism during platelet shape change induced by thrombin or platelet-activating factor: epinephrine does not induce phospholipase C activation or platelet shape change. *J. Biol. Chem.* **259:** 8286–8292.
13. Shaw, J. O., S. J. Klusick, and D. J. Hanahan. 1981. Activation of rabbit platelet phospholipase and thromboxane synthesis by 1-O-hexadecyl/octadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine (platelet activating factor). *Biochim. Biophys. Acta.* **663:** 222–229.
14. Shukla, S. D. 1985. Platelet activating factor-stimulated formation of inositol triphosphate in platelets and its regulation by various agents including Ca^{2+}, indomethacin, CV-3988, and forskolin. *Arch. Biochem. Biophys.* **240:** 674–681.
15. Berridge, M. J. 1984. Inositol trisphosphate and diacylglycerol as second messengers. *Biochem. J.* **220:** 345–360.
16. Emilsson, A., and R. Sundler. 1984. Differential activation of phosphatidylinositol deacylation and a pathway via diphosphoinositide in macrophages responding to zymosan and ionophore A23187. *J. Biol. Chem.* **259:** 3111–3116.
17. Ffeilschifter, J., A. Kurtz, and C. Bauer. 1986. Role of phospholipase C and protein kinase C in vasoconstrictor-induced prostaglandin synthesis in rat renal mesangial cells. *Biochem. J.* **234:** 125–130.
18. Naccache, P. H., M. M. Molski, M. Volpi, E. L. Becker, and R. I. Sha'afi. 1985. Unique inhibitory profile of platelet activating factor induced calcium mobilization, polyphosphoinositide turnover and granule enzyme secretion in rabbit neutrophils towards pertussis toxin and phorbol ester. *Biochem. Biophys. Res. Commun.* **130:** 677–684.
19. Tou, J-S. 1985. Platelet-activating factor promotes arachidonate incorporation into phosphatidylinositol and phosphatidylcholine in neutrophils. *Biochem. Biophys. Res. Commun.* **127:** 1045–1051.
20. Blank, M. L., E. A. Cress, T. Whittle, and F. Synder. 1981. In vivo metabolism of a new class of biologically active phospholipids: 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, a platelet activating-hypotensive phospholipid. *Life Sci.* **29:** 769–775.
21. Shukla, S. D., D. B. Buxton, M. S. Olson, and D. J. Hanahan. 1983. Acetylglyceryl ether phosphorylcholine: a potent activator of hepatic phosphoinositide metabolism and glycogenolysis. *J. Biol. Chem.* **258:** 10212–10214.
22. Creba, J. A., P. Downes, P. T. Hawkins, G. Brewster, R. H. Michell, and C. J. Kirk. 1983. Rapid breakdown of phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate in rat hepatocytes stimulated by vasopressin and other Ca^{2+}-mobilizing hormones. *Biochem. J.* **212:** 733–747.
23. Thomas, A. P., J. S. Marks, K. E. Coll, and J. R. Williamson. 1983. Quantitation and early kinetics of inositol lipid changes induced by vasopressin in isolated and cultured hepatocytes. *J. Biol. Chem.* **258:** 5716–5725.
24. Thomas, A. P., J. Alexander, and J. R. Williamson. 1984. Relationship between inositol polyphosphate production and the increase of cytosolic free Ca^{2+} induced by vasopressin in isolated hepatocytes. *J. Biol. Chem.* **259:** 5574–5584.
25. Fisher, R. A., S. D. Shukla, M. S. Debuysere, D. J. Hanahan, and M. S. Olson. 1984. The effect of acetylglyceryl ether phosphorylcholine on glycogenolysis and phosphatidylinositol 4,5-bisphosphate metabolism in rat hepatocytes. *J. Biol. Chem.* **259:** 8685–8688.
26. Charest, R., V. Prpic, J. H. Exoton, and P. F. Blackmore. 1985. Stimulation of inositol trisphosphate formation in hepatocytes by vasopressin, adrenalin and angiotensin II and its relationship to changes in cytosolic free Ca^{2+}. *Biochem. J.* **227:** 79–90.
27. Okayasu, T., K. Hoshii, K. Seyama, T. Ishibashi, and Y. Imai. 1986. Metabolism of platelet-activating factor in primary cultured adult rat hepatocytes by a new pathway involving phospholipase C and alkyl monooxygenase. *Biochim. Biophys. Acta.* **876:** 58–64.
28. Seglen, P. O. 1976. Preparation of isolated rat liver cells. *Methods Cell. Biol.* **13:** 29–83.
29. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–918.
30. Pieroni, G., and D. J. Hanahan. 1983. Metabolic behavior of acetyl glyceryl ether phosphorylcholine on interaction with rabbit platelets. *Arch. Biochem. Biophys.* **224:** 485–493.
31. Billah, M. M., and E. G. Lapetina. 1982. Formation of lysophosphatidylinositol in platelets stimulated with thrombin or ionophore A23187. *J. Biol. Chem.* **257:** 5196–5200.
32. Berridge, M. J., R. M. C. Dawson, C. P. Downes, J. P. Heslop, and R. F. Irvine. 1983. Changes in the levels of inositol phosphates after agonist-dependent hydrolysis of membrane phosphoinositides. *Biochem. J.* **212:** 473–482.
33. Sasaki, T., and H. Hasegawa-Sasaki. 1985. Breakdown of phosphatidylinositol 4,5-bisphosphate in a T-cell leukaemia line stimulated by phytohaemagglutinin is not dependent on Ca^{2+} mobilization. *Biochem. J.* **227:** 971–979.
34. Monaco, M. E. 1982. The phosphatidylinositol cycle in WRK-1 cells. Evidence for a separate, hormone-sensitive phosphatidylinositol pool. *J. Biol. Chem.* **257:** 2137–2139.
35. Kawaguchi, H., and H. Yasuda. 1986. Effect of platelet-activating factor on arachidonic acid metabolism in renal epithelial cells. *Biochim. Biophys. Acta.* **875:** 525–534.
36. Kawaguchi, H., and H. Yasuda. 1986. Platelet-activating factor stimulates prostaglandin synthesis in cultured cells. *Hypertension.* **8:** 192–197.
37. Buxton, D. B., R. A. Fisher, D. J. Hanahan, and M. S. Olson. 1986. Platelet-activating factor-mediated vasoconstriction and glycogenolysis in the perfused rat liver. *J. Biol. Chem.* **261:** 644–649.
38. Gray, N. C. C., and K. P. Strickland. 1982. The purification and characterization of a phospholipase A_2 activity from the 106,000 × *g* pellet (microsomal fraction) of bovine brain acting on phosphatidylinositol. *Can. J. Biochem.* **60:** 108–117.