0026-895X/88/060793-07$02.00/0

MOLECULAR PHARMACOLOGY, 34:793-799

Platelet-Activating Factor Stimulates Arachidonic Acid Metabolism in Rat Liver Cells (C-9 Cell Line) by a Receptor-Mediated Mechanism

LAWRENCE LEVINE

Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254

Received June 27, 1988; Accepted September 6, 1988

SUMMARY

Platelet activating factor (PAF) stimulated production of prostaglandin (PG) I_2, PGE_2, and $PGF_{2\alpha}$ by rat liver cells (the C-9 cell line); as little as 0.2 nM PAF was effective. *Enantio*-PAF was 1000-fold less effective. Lyso-PAF, at levels ranging from 0.1 to 1.0 μM, did not stimulate PGI_2 production. The synthesis of PGI_2 was essentially complete in 10 min. The stimulation by PAF of PGI_2 production was inhibited by the PAF antagonists L-659,989, kadsurenone, L-652,731, and BN 52021; the values for 50% inhibition (IC_{50}) were 0.02, 0.19, 0.21, and 0.73 μM, respectively. The antagonists L-659,989 and BN 52021 had no effect on the levels of 6-keto-$PGF_{1\alpha}$ stimulated by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), palytoxin, melittin, the Ca^{2+} ionophore-A-23187, colchicine, transforming growth factor α, or exogenous arachidonic acid. The effect of PAF on arachidonic acid metabolism was inhibited by prior exposure of the cells to PAF. Prior treatment of the rat liver cells at 37° with the TPA-type tumor promoters TPA, teleocidin, and aplysiatoxin, as well as with the second stage tumor promoter mezerein, all of which activate the Ca^{2+}/phospholipid-dependent protein kinase (protein kinase C), resulted not only in homologous desensitization to the TPA-type tumor promoters and mezerein, but also in heterologous desensitization to PAF. Stimulation of PGI_2 production by palytoxin, the Ca^{2+} ionophore A-23187, or exogenous arachidonic acid was not inhibited by such prior treatments with the TPA-type tumor promoters. Prior treatment of the cells at 37° for 30 min with the non-TPA-type tumor promoters okadaic acid or palytoxin, both of which do not activate protein kinase C, did not result in heterologous desensitization to PAF.

The pharmacological and physiological activities initiated by PAF [1-*O*-octadecyl- (or hexadecyl)-(2*R*)-acetyl-glycerol-3-phosphorylcholine]) have been the subject of several recent reviews (1–4). Its activities are manifold, which is not surprising given the multiple effects of PAF even on a single cell type. One such effect is stimulation of arachidonic acid metabolism. PAF stimulates arachidonic acid mobilization in human and rabbit polymorphonuclear leukocytes (5–7), guinea pig peritoneal macrophages (8), Swiss mouse 3T3 fibroblasts (9), and human endothelial cells (10). Based on (i) the low concentrations required to initiate biological responses, (ii) homologous desensitization, (iii) the demonstration that the biological responses are specific for the naturally occurring stereoisomer (*R*), and (iiii) specific inhibition by PAF antagonists, PAF activity is thought to be receptor-mediated (11). PAF receptors have been detected on rat liver plasma membranes (12). Judging from the multiple effects of PAF *in vivo* (1–4), many cells may contain specific PAF receptors, the occupany of which could stimulate arachidonic acid metabolism. The pathology would then depend, in part, on the deesterification reactions coupled to cyclooxygenase and lipoxygenase activities and, subsequently, the diverse biological activities of the cyclooxygenase and lipoxygenase products.

Several cells in culture were tested for their response to PAF. Only one cell line, a C-9 rat liver cell line, was stimulated by PAF to metabolize arachidonic acid.

Experimental Procedures

Materials. PAF(C-16, C-18) was purchased from Calbiochem-Behring Corp. (La Jolla, CA) and Sigma Chemical Co. (St. Louis, MO). Lyso-PAF (C-16) and the isomer of PAF, *enantio*-PAF, were purchased from Sigma. All reagents were stored at −20° as concentrated solutions in DMSO or chloroform and diluted into MEM for experiments. The chloroform or DMSO was diluted at least 1/1000, a concentration that has no effect on arachidonic acid metabolism in any of the cell lines used in our experiments. Aplysiatoxin, teleocidin, palytoxin, and okadaic acid were gifts from Dr. Hirota Fujiki, National Cancer Institute,

Send reprint requests to: Lawrence Levine, Department of Biochemistry, Brandeis University, Waltham, MA 02254.

L.L. is an American Cancer Society Research Professor of Biochemistry (Award PRP-21). This is Publication 1667 of the Department of Biochemistry, Brandeis University, Waltham, MA 02254.

ABBREVIATIONS: PAF, platelet activating factor; DMSO, dimethylsulfoxide; MEM, minimal essential medium; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGI_2, prostaglandin I_2; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; RIA, radioimmunoassay.

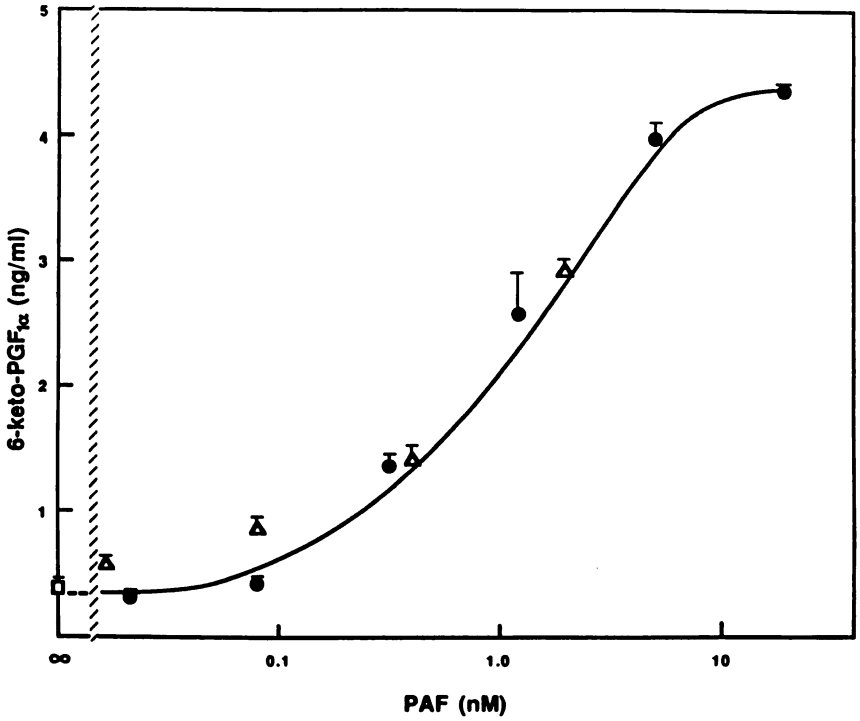

Fig. 1. Stimulation of 6-keto-PGF$_{1\alpha}$ production by increments of PAF. Rat liver cells (4×10^5/35-mm dish) were incubated at 37° with PAF for 20 hr, at which time the culture fluids were collected and assayed by RIA. The data △, ● are from two separate experiments and represent the mean ± standard error obtained with three culture dishes. In the absence of PAF (□), 0.41 ng of 6-keto-PGF$_{1\alpha}$/ml was found.

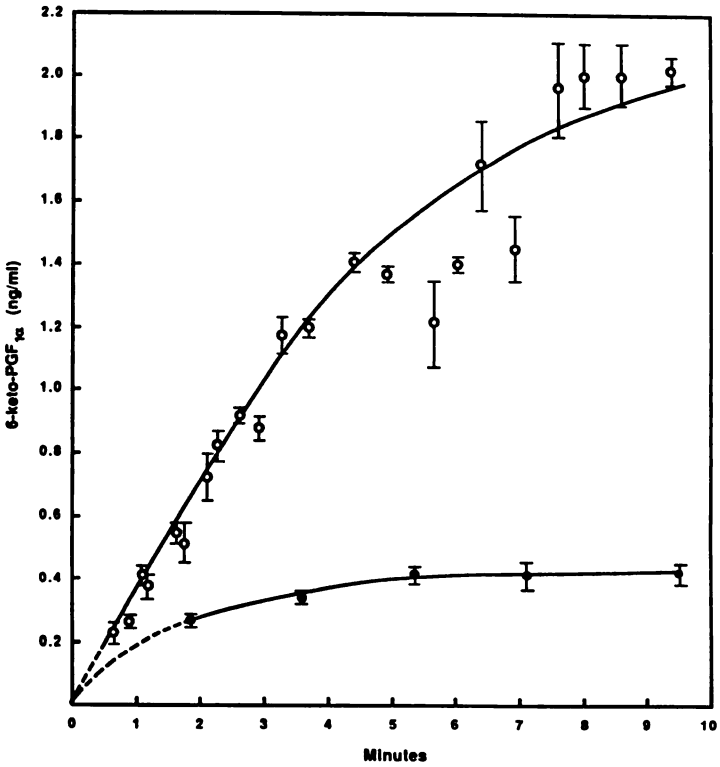

Fig. 2. Effect of time on the production of 6-keto-PGF$_{1\alpha}$ after addition of 1.9 nM PAF. The rat liver cells (4×10^6/35 mm dish) were incubated for the indicated times in 1.9 nM PAF (○) or MEM (●), at which time the culture fluids were collected and assayed by RIA. The data represent the mean ± standard error for three dishes.

Tokyo, Japan. The PAF antagonists were gifts from Merck Sharp & Dohme, (Rahway, NJ) (kadsurenone, L-652,731, and L-659,989) and Dr. P. Braquet, Institut Henri Beaufour Research Laboratories, Le Plessis Robinson, France (BN 52021). Recombinant transforming growth factor α (human) was a gift from Genentech (South San Francisco, CA). All the other reagents were obtained from Sigma.

Culture and cell lines. Cell lines were maintained at 37° in an atmosphere of 95% air/5% CO_2 in 100-mm culture dishes (Falcon, Cockeysville, MD) in Eagle's MEM (GIBCO Laboratories, Grand Island, NY) supplemented with 10% (v/v) fetal bovine serum. Bovine aorta endothelial cells were isolated as previously described (13, 14) and their identity was authenticated by the cobblestone appearance of confluent cultures under light microscopy (13) and positive immunofluorescent staining for Factor VIII (15). This cell line was obtained from Dr. K. C. Hayes, Department of Biology, Brandeis University. The C-9 rat liver cell line, the bovine aorta endothelial line (CPAE), the WEHI-1, the RBL-1, the normal rat kidney, and the Kirsten virus-transformed rat kidney cell lines were obtained from the American Type Culture Collection (Rockville, MD). Newborn rat cultured keratinocytes were isolated and grown as previously described (16). Their authenticity as keratinocytes was confirmed by biochemical and immunological detection of cytokeratines and filagrin, proteins unique to

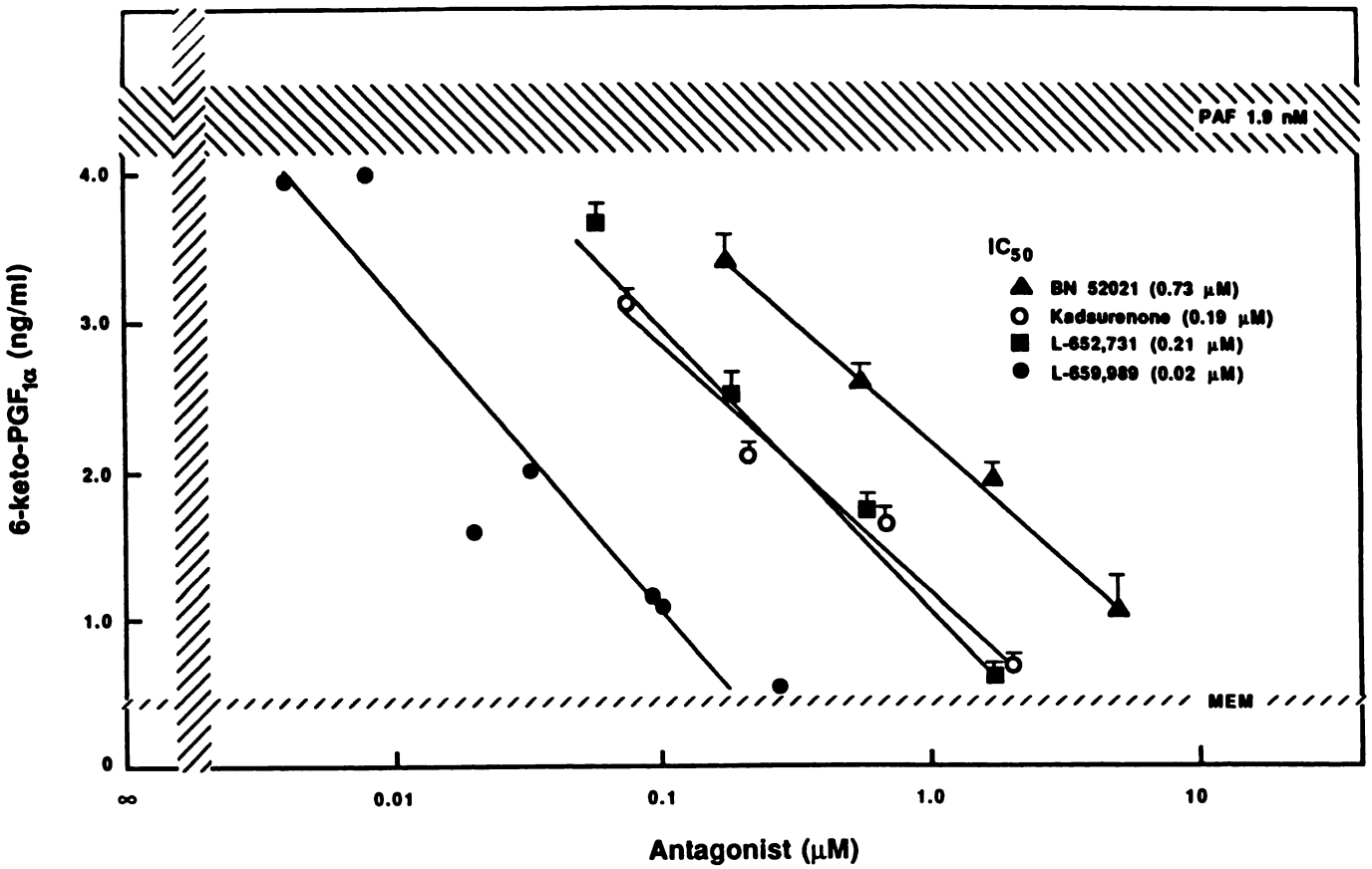

Fig. 3. Production of 6-keto-$PGF_{1\alpha}$ after incubation with 1.9 nM PAF in the presence of BN 52021 (▲), kadsurenone (○), L-652,731 (■), and L-659,989 (●). The data for BN 52021, kadsurenone, and L-652,731 are the mean ± standard errors for three culture dishes whereas the data for L-659,989 represent the means of duplicate analyses in two separate experiments. The values agreed within 10% of their means.

squamous epithelia. The bovine aortic smooth muscle cells were cultured and characterized as reported (17). The porcine endothelial cells were obtained from Dr. Michael Moskowitz, Department of Neurosurgery, Massachusetts General Hospital (Boston, MA).

All experiments were done on cells that had undergone at least 25 passages. One day before experiments, subconfluent cultures were treated with 0.25% trypsin-EDTA (GIBCO) and seeded onto 35 mm culture dishes (Falcon). The plating densities for the endothelial cells, the keratinocytes, and the smooth muscle cells, as determined by counting cells in a hemocytometer chamber, varied from 0.5 to 4×10^5/35-mm dish; the density for the C-9 cells was 4×10^5/35-mm dish. These freshly seeded cultures were incubated overnight to allow for cell attachment. The plating efficiencies for all of the cells in this medium were not determined. At subconfluent cell densities, production of arachidonic acid metabolites is maximal (18, 19) and the products are not further metabolized to 15-keto- or 13,14-dihydro-15-keto-compounds (20). The cells were then washed twice with MEM and incubated in MEM in the absence and presence of the modulators. At designated times, culture fluids were collected and analyzed by RIA (21).

Procedure for desensitization experiments. The rat liver cells (4×10^5/35-mm dish) were incubated at 37° for the designated times with the different reagents. The cells then were washed twice with 1.0 ml of MEM and incubated with the specified reagents for various periods of time. Cells incubated with MEM alone for the same periods of time served as controls. For experiments to test for desensitization by prior exposure to PAF at 0°, the dishes containing the cells to be used were placed on a block of ice for 5 min, at which time they were washed with 0° MEM and incubated with and without 0° PAF on the block of ice for 2.5 min. They then were washed twice with the 0° MEM. After the second wash, the dishes containing the second 1.0 ml of 0° MEM were removed from the ice block and 37° MEM and 37° PAF were added and the cells were incubated at 37° for various periods of time.

RIA. RIA was performed as previously described (21) with antibodies of known specificities. The limits of sensitivity for the PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and thromboxane B_2 immune systems are 0.009, 0.012, 0.018, and 0.005 ng/ml, respectively.

Presentation of data. The absolute levels of arachidonic acid metabolites produced by cells in culture varied from experiment to experiment, but the relative differences in the levels of cyclooxygenase metabolites produced by the treated compared with control cells remained reproducible in each experiment. All experiments were performed more than twice.

Results

Several cells, rat liver (the C-9 line), porcine aorta endothelial, bovine aorta endothelial, bovine aorta smooth muscle, rat keratinocytes (the NBR line), normal rat kidney, Kirsten virus-transformed rat kidney, mouse lymphoma (the WEHI-3 line), and rat basophil leukemia (the RBL-1 line), were tested for their response to PAF at levels ranging from 1 nM to 1 μM. All of the cells have undergone at least 25 passages and, therefore, may have lost their responsiveness to some receptor-binding agonists. Although all of the cells were stimulated to metabolize arachidonic acid by the Ca^{2+}ionophore A-23187, only one of the multiple passaged cells' arachidonic acid metabolism was stimulated by treatment with PAF. PAF stimulated production of PGI_2, measured as its stable hydrolytic product 6-keto-$PGF_{1\alpha}$, by rat liver cells (the C-9 cell line); as little as 0.2 nM PAF was

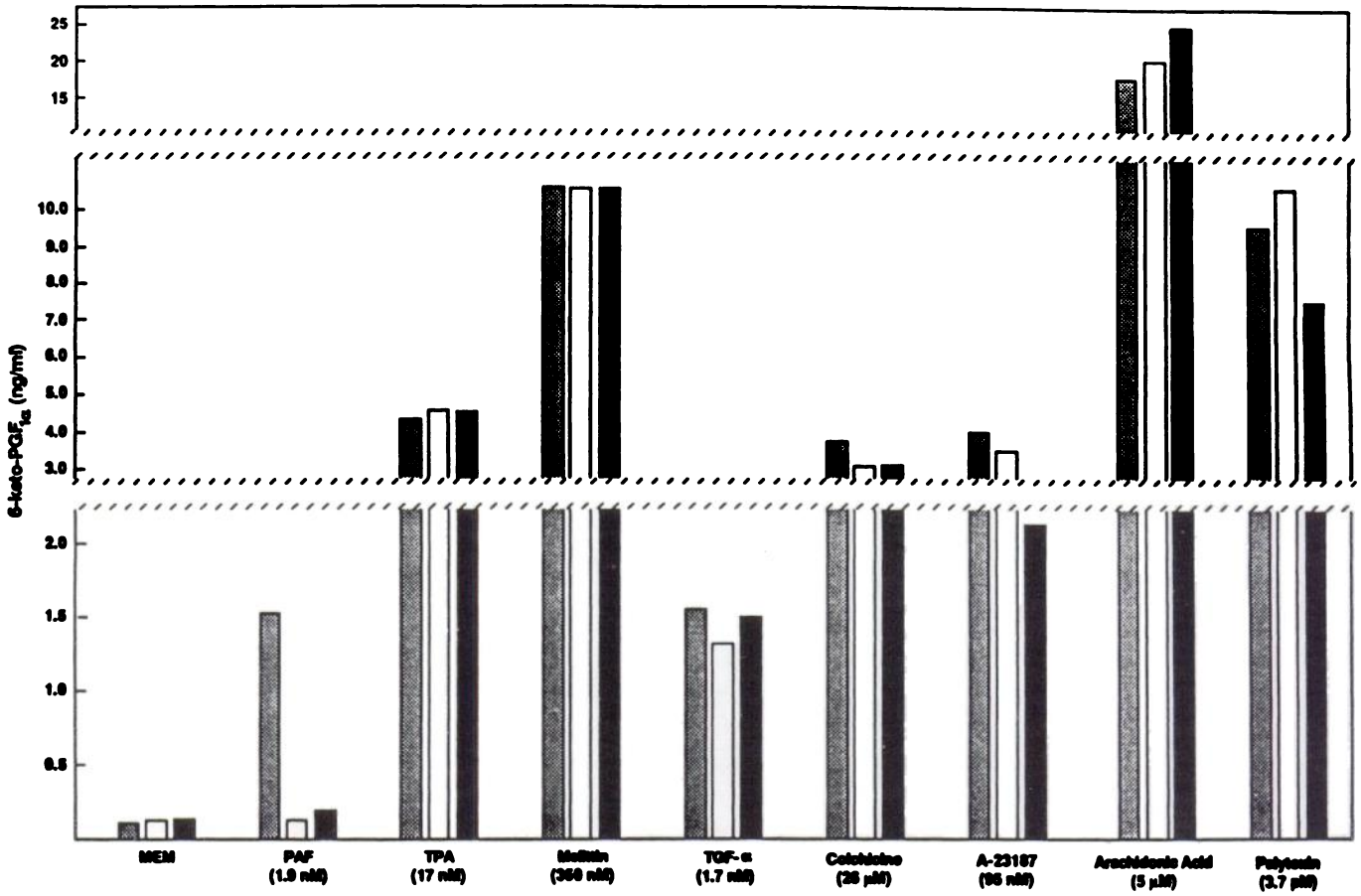

Fig. 4. The effect of 15 μM BN 52021 (■) and 2 μM L-659,989 (□) on 6-keto-$PGF_{1\alpha}$ production stimulated by PAF, TPA, melittin, transforming growth factor α (TGF-α), colchicine, A-23187, arachidonic acid, and palytoxin. The rat liver cells (4×10^5/35-mm dish) were incubated at 37° in the presence of the various stimulants in the presence and absence (■) of antagonists for 20 hr, at which time the culture fluids were collected and assayed by RIA. The data represent the averages of duplicate determinations. The values agreed within 10% of these averages.

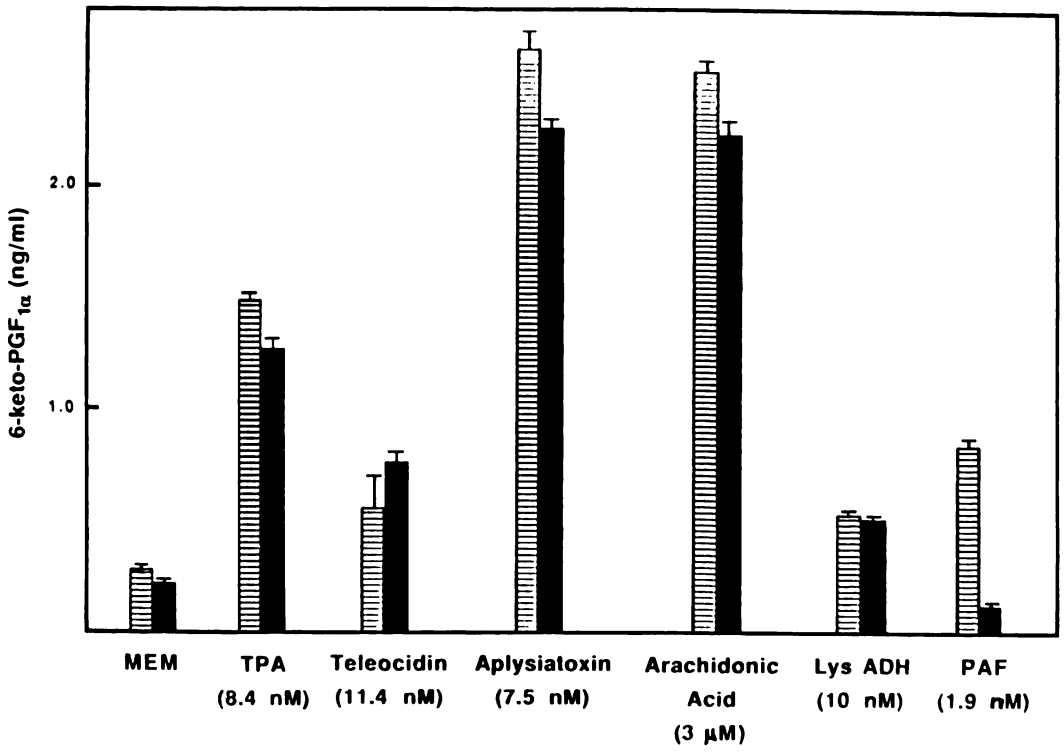

Fig. 5. The effect of preincubation of the rat liver cells with 190 nM PAF for 30 min at 37° on the stimulation of 6-keto-$PGF_{1\alpha}$ production, after removal of free PAF, stimulated by TPA, teleocidin, aplysiatoxin, arachidonic acid, vasopressin (*Lys ADH*), and PAF. The rat liver cells (4×10^5/35 mm dish) were incubated for 30 min at 37° in the presence (■) and absence (■) of PAF, at which time they were washed twice with MEM and reincubated for 20 hr in the presence of the various agonists. The culture fluids were analyzed for 6-keto-$PGF_{1\alpha}$ production by RIA. The data represent the mean ± standard error for three dishes.

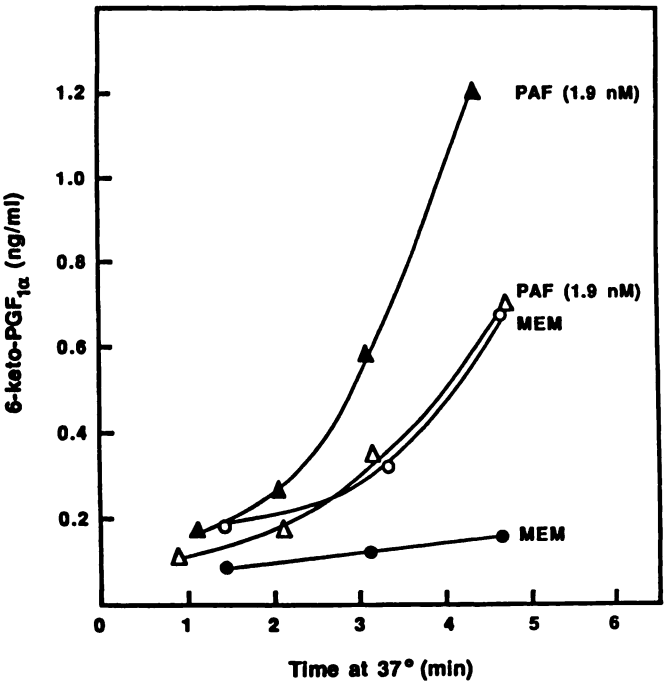

Fig. 6. Effect of preincubation of the cells with 19 nM PAF (*open symbols*) and MEM (*closed symbols*) at 0° for 2.5 min on the subsequent 6-keto-$PGF_{1\alpha}$ production after two washes of the cells at 0° followed by incubation at 37° in the presence of 1.9 nM PAF (*triangles*) and MEM (*circles*). The rat liver cells (4×10^5/35-mm dish) were kept at 0° (on an ice block) for 5 min, at which time 1.0 ml of prechilled MEM or 1.9 nM PAF was added. The cells were kept at 0° for 2.5 min, washed twice with prechilled MEM, and then reincubated at 37° in the presence of 1.9 nM PAF (*triangles*) or MEM (*circles*). At various times of incubation at 37°, culture fluids were collected and analyzed for 6-keto-$PGF_{1\alpha}$ by RIA. The data represent the averages of two dishes. The values agreed within 20% of these averages.

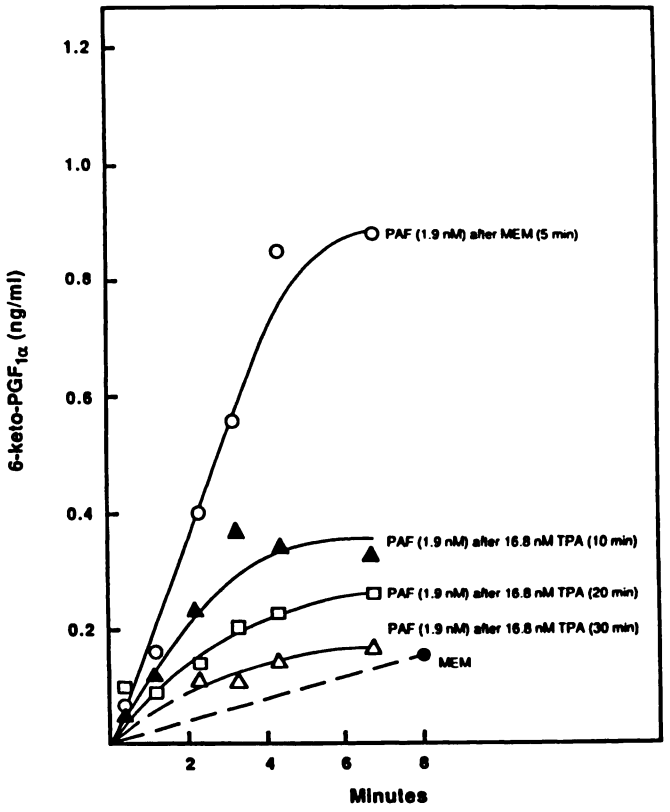

Fig. 7. Effect of preincubation of the rat liver cells (4×10^5/35-mm dish) with 16.8 nM TPA for 10 (▲), 20 (□), and 30 (△) min at 37° or with MEM for 5 min (○) on stimulation, after two washes of the cells with MEM, by 1.9 nM PAF. The values represent the averages of two dishes. The values agreed within 20% of these averages.

effective (Fig. 1). *Enantio*-PAF was 1000-fold less effective and lyso-PAF, when tested at levels ranging from 0.1 to 1.0 μM, did not affect 6-keto-$PGF_{1\alpha}$ production by these cells. C-9 cells metabolize arachidonic acid by the cyclooxygenase, not the lipoxygenase, pathway and greater than 90% of the cyclooxygenase products are PGI_2, with lesser, but measurable, amounts of PGE_2 and $PGF_{2\alpha}$ (22). Production of PGE_2 and $PGF_{2\alpha}$ also was stimulated by 1.9 nM PAF, suggesting that the stimulation was occurring at or before the cyclooxygenase reaction. The stimulation by 1.9 nM PAF was essentially complete in 10 min (Fig. 2). Even when measured at 15 min, 30 min, 60 min, 120 min, 240 min, and 20 hr, the difference between the 6-keto-$PGF_{1\alpha}$ produced by 1.9 nM PAF and MEM was essentially the same as that measured after 10 min (data not shown). As expected, indomethacin and minoxidil inhibited the production of 6-keto$PGF_{1\alpha}$, most likely by blocking synthesis of PGG_2 or PGI_2, respectively (23, 24). The 50% inhibition levels were 1.1 and 600 μM, respectively. Inasmuch as stimulation of prostaglandin or leukotriene production usually is associated with the increased availability of free arachidonic acid (25, 26), PAF is probably stimulating deesterification of arachidonic acid-containing phospholipids.

Four PAF antagonists, the recently described L-659,989 (27), kadsurenone, L-652,731, and BN 52021 dose-dependently inhibited the PAF (1.9 nM)-stimulated 6-keto-$PGF_{1\alpha}$ production; the IC_{50} values were 0.02, 0.19, 0.21, and 0.73 μM, respectively (Fig. 3). L-659,989 and BN 52021, when tested at 2 and 15 μM, respectively, did not affect 6-keto-$PGF_{1\alpha}$ production stimulated by TPA, palytoxin, recombinant transforming growth factor-α, colchicine, the Ca^{2+}ionophore A-23187, melittin, or exogenous arachidonic acid (Fig. 4).

After a 30-min exposure of the rat liver cells to 190 nM PAF and removal of any unbound PAF, stimulation of 6-keto-$PGF_{1\alpha}$ by 1.9 nM PAF was lost but the stimulation of 6-keto-$PGF_{1\alpha}$ production by the tumor promoters TPA, teleocidin, and aplysiatoxin, the hormone lysine^8-vasopressin, and exogenous arachidonic acid were not (Fig. 5). This homologous down-regulation was seen after as little as 2.5 min of exposure to 19 nM PAF at 37°. Even exposure of the cells to PAF (19 nM) at 0° for 2.5 min resulted in down-regulation when the cells were again exposed to PAF (1.9 nM) at 37°. The cells exposed to 19 nM PAF for 2.5 min at 0° responded no more in the presence of 1.9 nM PAF than in its absence (Fig. 6). However, these cells were affected, as measured by their stimulated 6-keto-$PGF_{1\alpha}$ production at 37°, even in the absence of added PAF.

Exposure of the rat liver cells to the tumor promoter TPA (16.8 nM) desensitized the response of the cells to 1.9 nM PAF, and this desensitization was pronounced even after 15-min exposure (Fig. 7). Exposure of the cells to the TPA-type tumor promoters teleocidin and aplysiatoxin (28), and the second-stage tumor promoter mezerein (29) for 60 min, but not exposure to the non-TPA-type tumor promoters okadaic acid (30) and palytoxin (28), also led to desensitization of stimulation of 6-keto-$PGF_{1\alpha}$ production by PAF (1.9 nM). Such exposure of the cells to the TPA-type tumor promoters TPA, teleocidin, and aplysiatoxin, as well as to mezerein but not PAF or the

non-TPA-type tumor promoters okadaic acid and palytoxin, although it did lead to homologous and heterologous desensitization of stimulated 6-keto-$PGF_{1\alpha}$ production by TPA, teleocidin and aplysiatoxin, resulted in an amplification of 6-keto-$PGF_{1\alpha}$ production upon incubation with 6.7 nM ionomycin.

Discussion

Rat liver cells (the C-9 cell line) responded to PAF as measured by arachidonic acid metabolism; other cells did not. Probably several of the nonresponsive cells had lost their specific receptor during multiple passages. (i) PAF, at subnanomolar levels, stimulated arachidonic acid metabolism by rat liver cells (the C-9 cell line); (ii) *enantio*-PAF was 1000-fold less effective than the natural isomer; (iii) the response of PAF was inhibited by prior exposure of the cells to PAF, even prior exposure at 0°; and (iv) its stimulation was specifically inhibited by PAF antagonists. The stimulation therefore appears to be mediated by occupany of a PAF-receptor. With respect to point iii, the desensitization seen in the data presented in Figs. 5 and 6 could reflect the saturation of receptors by PAF and the lack of its removal by the washing procedure. Such a possibility can also account for the increased 6-keto-$PGF_{1\alpha}$ production seen after the cells were treated with PAF at 0°, washed, and then incubated at 37° in MEM (Fig. 6). Elucidation of the mechanism of such desensitization must await development of procedures for quantitation of PAF and its metabolites at levels used in this study and measurement of the specific binding of PAF to these cells.

TPA-type tumor promoters desensitized the stimulation of 6-keto-$PGF_{1\alpha}$ production by PAF. TPA has been shown to reduce binding of EGF to its receptor (31), to decrease internalization of EGF (32), and to potentiate the mitogenic activity of EGF in quiescent cells (33, 34). TPA and the TPA-type tumor promoters teleocidin and aplysiatoxin block EGF-stimulated tyrosine-specific phosphorylation of the EGF receptor in A431 cells, and this block is associated with the reduction of high affinity binding of EGF to A431 cells (35). One explanation for the heterologous desensitization reported in our studies is that the TPA-type tumor promoters are down-regulating the activity of PAF by reducing its binding. This should be amenable to experimentation, but we have been unsuccessful in developing a PAF binding assay on our cells; the magnitude of the nonspecific binding of PAF makes such analyses questionable. The possibility that one of the biochemical reaction products of the TPA-type tumor promoters is inhibiting one of the biochemical reaction products of PAF, a reaction product distal to receptor occupany, cannot be ruled out. PAF receptor interaction in platelets leads to increased phosphoinositide turnover, phosphatidic acid formation (9, 36–39), Ca^{2+} mobilization (40–43), and phosphorylation of the M_r 20,000 myosin light chain and the M_r 47,000 protein, the substrate for the Ca^{2+}/phospholipid-dependent protein kinase [protein kinase C (44)]. Thus, a highly speculative scenario in which the TPA-type tumor promoters and the second-stage tumor promoter mezerein activate protein kinase C to phosphorylate serine and/or threonine in a PAF pathway reaction product and negatively modulate the stimulation of arachidonic acid mobilization by PAF is possible. The phosphorylations in this scenario are based upon biochemical events that are associated with the inhibition of EGF binding (35) or stimulation of insulin binding (45, 46) by TPA. The TPA-type tumor promoters TPA, teleocidin, and aplysiatoxin and the second-stage tumor promoter mezerein activate the Ca^{2+}/phospholipid-dependent kinase, protein kinase C (28, 47); they heterologously desensitized the stimulation of arachidonic acid metabolism by PAF. The non-TPA tumor promoters okadaic acid and palytoxin do not activate protein kinase C (28, 30); they did not heterologously desensitize the stimulation of arachidonic acid metabolism by PAF.

Acknowledgments

I wish to thank Nancy Worth and Xiaoying Chen for their technical assistance and Inez Zimmerman for preparation of the manuscript.

References

1. Braquet, P., L. Touqui, T. Y. Shen, and B. B. Vargaftig. Perspectives in platelet-activating factor research. *Pharmacol. Rev.* **39**:97–145 (1987).
2. Snyder, F., ed. *Platelet-Activating Factor and Related Lipid Mediators.* Plenum Press, New York (1987).
3. Houlihan, W. J., and R. N. Saunders. Platelet activating factor (PAF): a biologically active ether phospholipid. *Triangle* **25**:97–103 (1986).
4. Snyder, F. Chemical and biochemical aspects of platelet activating factor: a novel class of acylated ether-linked choline-phospholipids. *Med. Res. Rev.* **5**:107–140 (1986).
5. Chilton, F. H., J. T. O'Flaherty, C. E. Walsh, M. J. Thomas, R. L. Wykle, L. R. DeChatelet, and B. M. Waite. Platelet activating factor: stimulation of the lipoxygenase pathway in polymorphonuclear leukocytes by 1-*O*-alkyl-2-*O*-acetyl-*sn* glycero-3-phosphocholine. *J. Biol. Chem.* **257**:5402–5407 (1982).
6. Lin, A. H., D. R. Morton, and R. R. Gorman. Acetyl glyceryl ether phosphorylcholine stimulates leukotriene B_4 synthesis in human polymorphonuclear leukocytes. *J Clin Invest* **70**:1058–1065 (1982).
7. Ingraham, L. M., T. D. Coates, J. M. Allen, C. P. Higgins, R. L. Baehner, and L. A. Boxer. Metabolic, membrane, and functional responses of human polymorphonuclear leucocytes to platelet-activating factor. *Blood* **59**:1259–1266(1987).
8. Hartung, H. P. Acetyl glyceryl ether phosphorylcholine (platelet-activating factor) mediates heightened metabolic activity in macrophages: studies on PGE, TXB_2 and O_2 production, spreading, and the influence of calmodulin-inhibitor W-7. *FEBS Lett.* **160**:209–212 (1983).
9. Kawaguchi, H., and H. Yasuda. Platelet-activating factor stimulates phospholipase in quiescent Swiss mouse 3T3 fibroblast. *FEBS Lett.* **176**:93–96 (1984).
10. D'Humieres, S., F. Russo-Marie, and B. B. Vargaftig. PAF-acether-induced synthesis of prostacyclin by human endothelial cells. *Eur. J. Pharmacol.* **131**:13–19 (1986).
11. Godfroid, J. J., and P. Braquet. PAF-acether specific binding sites. 1. Quantitative SAR study of PAF-acether isosteres. *Trends Pharmacol. Sci.* **7**:368–373 (1986).
12. Huang, S.-B. Specific receptor sites for platelet activating factor on rat liver plasma membranes. *Arch. Biochem. Biophys.* **257**:339–344 (1987).
13. Alderson, L. M., G. Endemann, S. Lindsey, A. Pronczuk, R. L. Hoover, and K. C. Hayes. LDL enhances monocyte adhesion to endothelial cells *in vitro*. *Am. J. Pathol.* **123**:334–342 (1986).
14. Booyse, F. M., B. J. Sedlak, and M. E. Rafelson. Culture of arterial endothelial cells: characterization of growth of bovine aortic cells. *Thromb. Diath. Haemorrh.* **34**:825–839 (1975).
15. Jaffe, E. A., L. W. Hoyer, and R. Nachman. Synthesis of antihemophilic factor antigen by cultured human endothelial cells. *J. Clin. Invest.* **52**:2757–2764 (1973).
16. Kvedar, J., and L. Levine. Modulation of arachidonic acid metabolism in a cultured newborn rat keratinocyte cell line. *J. Invest. Dermatol.* **88**:124–129 (1987).
17. Coughlin, S. R., M. A. Moskowitz, B. R. Zetter, H. N. Antoniades, and L. Levine. Platelet-dependent stimulation of prostacyclin synthesis by platelet-derived growth factor. *Nature* (*Lond.*) **288**:600–602 (1980).
18. Hong, S. L., C. M. Wheless, and L. Levine. Elevated prostaglandin synthetase activity in methylcholanthrene-transformed mouse BALB 3T3. *Prostaglandins* **13**:271–279 (1977).
19. Rice, R. H., and L. Levine. Melittin-stimulated arachidonic acid metabolism by cultured malignant human epidermal keratinocytes. *Biochem. Biophys. Res. Commun.* **124**:303–307 (1984).
20. Alam, I., K. Ohuchi, and L. Levine. Determination of cyclooxygenase products and prostaglandin metabolites using high-pressure liquid chromatography and radioimmunoassay. *Anal. Biochem.* **93**:339–345 (1979).
21. Levine, L. Measurement of arachidonic acid metabolites by radioimmunoassay, in *Manual of Clinical Laboratory Immunology* (J. L. Fahey, H. Friedman, and N. R. Rose, eds.), 3rd Ed. American Society for Microbiology, Washington, 685–691 (1986).
22. Rigas, A., and L. Levine. Arachidonic acid metabolism by rat liver cells (the C-9 cell line). *J. Pharmacol. Exp. Ther.* **231**:230–235 (1984).
23. Vane, J. R. Inhibition of prostaglandin synthetase as a mechanism of action of aspirin drugs. *Nature New Biol.* **231**:232–235 (1971).
24. Kvedar, J. D., H. P. Baden, and L. Levine. Minoxidil selectively inhibits

prostacyclin production by cells in culture. *Biochem. Pharmacol.* **37**:867–874 (1988).
25. Lands, W.E.M., and B. Samuelsson. Phospholipid precursor of prostaglandins. *Biochim. Biophys. Acta* **164**:426–429 (1968).
26. Irvine, R. F. How is the level of free arachidonic acid controlled in mammalian cells? *Biochem. J.* **204**:3–16 (1982).
27. Ponpipom, M. M., S.-B. Hwang, T. W. Doebber, J. J. Acton, A. W. Alberts, T. Biftu, D. R. Brooker, R. L. Bugianesi, J. C. Chabala, N. L. Gamble, D. W. Graham, M.-H. Lam, and M. S. Wu. (±)-*trans*-2-(3-Methoxy-5-methylsulfonyl-4-propoxyphenyl)-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran (L-659,989), a novel, potent PAF receptor antagonist. *Biochem. Biophys. Res. Commun.* **150**:1213–1220 (1988).
28. Fujiki, H., and T. Sugimura. New classes of tumor promoters: teleocidin, aplysiatoxin and palytoxin. *Adv. Cancer Res.* **49**:223–264 (1987).
29. Slaga, T. J., S. M. Fischer, K. Nelson, and G. L. Gleason. Studies of the mechanism of skin tumor promotion: evidence for several stages in promotion. *Proc. Natl. Acad. Sci USA* **77**:3659–3663 (1980).
30. Suganuma, M., H. Fujiki, H. Suguri, S. Yoshizawa, M. Hirota, M. Nakayasu, M. Ojika, K. Wakamatsu, K. Yamada, and T. Sugimura. Okadaic acid: a new non-12-*O*-tetradecanoylphorbol-13-acetate type tumor promoter. *Proc. Natl. Acad. Sci. USA* **85**:1768–1771 (1988).
31. Lee, L.-S., and I. B. Weinstein. Tumor-promoting phorbol esters inhibit binding of epidermal growth factor to cellular receptors. *Science* (*Wash. D. C.*) **202**:313–315 (1978).
32. Magun, B. E., L. M. Matrisian, and G. T. Bowden. Epidermal growth factor: ability of tumor promoters to alter its degradation, receptor affinity and receptor number. *J. Biol. Chem.* **255**:6373–6381 (1980).
33. Dicker, P. E., and E. Rozengurt. Stimulation of DNA synthesis by tumour promoter and pure mitogenic factors. *Nature* (*Lond.*) **276**:723–726 (1978).
34. Frantz, C. N., C. D. Stiles, and C. D. Schere. The tumor promoter 12-*O*-tetradecanoyl-phorbol-13-acetate enhances the proliferative response of BALB/c-3T3 cells to hormonal growth factors. *J. Cell. Physiol.* **100**:413–424 (1979).
35. Friedman, B., A. R. Frackelton, A. H. Ross, J. M. Connors, H. Fujiki, T. Sugimura, and M. R. Rosner. Tumor promoters block tyrosine-specific phosphorylation of the epidermal growth factor receptor. *Proc. Natl. Acad. Sci. USA* **81**:3034–3038 (1984).
36. Mauco, G., H. Chap, and L. Douste-Blazy. Platelet activating factor (PAF-acether) promotes an early degradation of phosphatidylinositol-4,5-biphosphate in rabbit platelets. *FEBS Lett.* **153**:361–365 (1983).
37. Shukla, S. D., and D. J. Hanahan. 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 (1982).
38. Lapetina, E. G., and F. L. Siegel. Shape changes induced in human platelets by platelet-activating factor. *J. Biol. Chem.* **258**:7241–7244 (1982).
39. Shukla, S. D., D. B. Buxton, M. S. Olson, and D. J. Hanahan. Acetylglyceryl ether phosphorylcholine: a potent activator of hepatic phosphoinositide metabolism and glycogenolysis. *J. Biol. Chem.* **258**:10212–10214 (1983).
40. Shukla, S. S., and D. J. Hanahan. Acetylglyceryl ether phosphorylcholine (AGEPC, platelet-activating factor)-induced stimulation of rabbit platelets: correlation between phosphatidic acid level, $^{45}Ca^{2+}$ uptake and [^{3}H]serotonin secretion. *Arch. Biochem. Biophys.* **232**:458–466 (1984).
41. Lee, T.-C., B. Malone, M. L. Blank, and F. Snyder. 1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor) stimulates calcium influx in rabbit platelets. *Biochem. Biophys. Res. Commun.* **102**:1262–1268 (1981).
42. Clare, K. A., and M. C. Scrutton. The role of Ca^{2+} uptake in the response of human platelets to adrenaline and to 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor). *Eur. J. Biochem.* **140**:129–136 (1984).
43. Valone, F. H., and B. Johnson. Modulation of cytoplasmic calcium in human platelets by the phospholipid platelet-activating factor 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphorylcholine. *J. Immunol.* **134**:1120–1124 (1985).
44. Kloprogge, E., P. Hasselaar, G. Gorter, and J.-W. N. Akkerman. Stimulus-aggregation coupling in platelets activated with PAF-acether. *Biochem. Biophys. Res. Commun.* **883**:127–137 (1986).
45. Jacobs, S., N. E. Sayyoun, A. R. Saltiel, and P. Cuatracases. Phorbol esters stimulate the phosphorylation of receptors for insulin and somatomedin C. *Proc. Natl. Acad. Sci. USA* **80**:6211–6213 (1983).
46. Walaas, O., and S. I. Walaas. Second messengers identified for insulin? *Trends Pharmacol. Sci.* **9**:151–152 (1988).
47. Weinstein, I. B., J. Arcoleo, J. Backer, A. Jeffrey, W.-L. W. Hsiao, S. Gattono-Celli, P. Kirschmeier, and E. Okin. Molecular mechanisms of tumor promotion and multistage carcinogenesis, in *Cellular Interactions by Environmental Tumor Promoters* (H. Fujiki, E. Hecker, R. E. Moore, T. Sugimura, and I. B. Weinstein, eds.). Japan Scientific Societies Press, Tokyo/VNU Science Press, Utrecht, 59-74 (1984).