Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 3859–3862, June 1985
Medical Sciences

Phorbol myristate acetate inhibits thrombin-stimulated Ca^{2+} mobilization and phosphatidylinositol 4,5-bisphosphate hydrolysis in human platelets

(phorbol esters/phosphatidic acid/quin-2/secretion/protein kinase C)

GEORGE B. ZAVOICO*, STEPHEN P. HALENDA*, RAMADAN I. SHA'AFI†, AND MAURICE B. FEINSTEIN*

Departments of *Pharmacology and †Physiology, University of Connecticut Health Center, Farmington, CT 06032

Communicated by M. J. Osborn, February 8, 1985

ABSTRACT **The tumor-promoting phorbol diester 4β-phorbol 12-myristate 13-acetate (PMA) inhibited mobilization of intracellular Ca^{2+} in platelets by thrombin (also trypsin and 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphocholine). PMA was effective over the same concentration range that activates protein kinase C in intact platelets; IC_{50} vs. thrombin = 2 ng/ml, 3.4 nM: >90% inhibition at 10–20 ng/ml. Suppression of thrombin-induced Ca^{2+} mobilization was evident within 30 sec of pretreatment with PMA and was essentially complete by 6–10 min at 10–20 ng of PMA per ml. Thrombin-induced secretion was initially accelerated in the presence of PMA, but after 1 min it was progressively inhibited when Ca^{2+} mobilization was depressed by >60%. PMA did not inhibit Ca^{2+} mobilization or secretion caused by A23187. Thrombin-induced phosphatidylinositol 4,5-[^{32}P]bisphosphate breakdown and [^{32}P]phosphatidic acid production were also initially increased by PMA and then progressively depressed. Inhibition of thrombin-induced lipid metabolism required higher concentrations of PMA (IC_{50} = 10 ng/ml), and it was not overcome by A23187. 4α-Phorbol 12,13-didecanoate, which lacks the ability to activate protein kinase C, did not inhibit any responses to thrombin. These results suggest that activation of protein kinase C, which initially fosters secretion and aggregation, may subsequently exert negative feedback on the receptor-mediated mobilization of intracellular Ca^{2+} and the hydrolysis of phosphatidylinositol 4,5-bisphosphate.**

The stimulation of platelets to secrete and aggregate by agonists, such as thrombin and 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphocholine (PAF-acether), is associated with a rapid increase of the cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) (1, 2) and the hydrolysis of the phosphodiester bond of phosphatidylinositol 4,5-bisphosphate (PtdIns-4,5-P_2) (3, 4). The latter reaction is closely correlated with stimulus–response coupling in many cell types, including platelets (5). The products of this reaction, 1,2-diacylglycerol ($acyl_2Gro$) and *myo*-inositol 1,4,5-trisphosphate (Ins-1,4,5-P_3), appear to play important roles in various cellular activation processes. $Acyl_2Gro$ acts as a second messenger to stimulate Ca^{2+}/phosphatidylserine-dependent protein kinase C by increasing the affinity of the enzyme–lipid complex for Ca^{2+}, so that the enzyme can be activated even at resting levels of $[Ca^{2+}]_i$ (6). $Acyl_2Gro$ is subsequently metabolized by further hydrolysis to release arachidonic acid and by phosphorylation to form phosphatidic acid (PtdOH). The action of $acyl_2Gro$ on protein kinase C can be duplicated by tumor-promoting phorbol esters (7). Ins-1,4,5-P_3, on the other hand, has been implicated as a second messenger that causes the release of Ca^{2+} from intracellular storage sites in various cells (8, 9), including platelets (10), thereby providing a mechanism by which receptor-mediated activation of the hydrolysis of PtdIns-4,5-P_2 can potentially lead to elevation of $[Ca^{2+}]_i$.

The stimulation of protein kinase C by exogenous $acyl_2Gro$ or phorbol diesters is accompanied by slow and partial secretion from platelets (7) without a rise of $[Ca^{2+}]_i$ that is detectable by quin-2 (2). However, secretion is strikingly enhanced when $[Ca^{2+}]_i$ is slightly elevated by ionophore A23187 or by ionomycin concurrently with the activation of protein kinase C by exogenous $acyl_2Gro$ or 4β-phorbol 12-myristate 13-acetate (PMA) (11). Currently, the only known pathway that antagonizes receptor-linked increase of $[Ca^{2+}]_i$ (12–15), the hydrolysis of PtdIns-4,5-P_2 (16), and the formation of Ins-1,4,5-P_3 (17) is mediated by cyclic AMP. In this paper we report that PMA inhibits Ca^{2+} mobilization, breakdown of PtdIns-4,5-P_2, formation of PA, and secretion caused by thrombin. This suggests that the protein kinase C pathway can exert feedback inhibition on receptor-mediated platelet activation subsequent to its initial role in promoting secretion.

METHODS AND MATERIALS

Fresh platelet concentrates obtained from the Connecticut Red Cross Blood Center were centrifuged at 120 × *g* for 10 min to remove residual erythrocytes and the platelets were then sedimented at 1000 × *g* for 10 min. The platelet pellet was resuspended in about 25 ml of buffer A (10 mM sodium Pipes, pH 6.5/0.2 mM EGTA/135 mM NaCl/5 mM KCl/5.5 mM glucose) and then recentrifuged at 1000 × *g* for 10 min. The platelet pellet was resuspended with 15–20 ml of buffer B (10 mM sodium Hepes, pH 7.4/135 mM NaCl/5 mM KCl/5.5 mM glucose) and incubated for 30 min with 0.2 mCi (1 Ci = 37 GBq) of [^{32}P]orthophosphate and 10–15 μM quin-2 acetoxymethyl ester in dimethyl sulfoxide [final dimethyl sulfoxide concentration, 0.15% (vol/vol)] with gentle agitation in a water bath at 37°C. The platelets were then washed with buffer B containing 0.2 mg of bovine serum albumin per ml and resuspended in the same solution or in buffer C (buffer B with 0.2 mg of bovine serum albumin per ml/1 mM $CaCl_2/MgCl_2$) to obtain a final concentration of 1.5–2.6 × 10^9 platelets per ml.

$[Ca^{2+}]_i$ was measured, as described previously (12, 15), in quin-2-loaded platelets diluted to 10^8 platelets per ml in either (*i*) buffer B with bovine serum albumin, (*ii*) buffer C with or without $CaCl_2$, or (*iii*) buffer C with 2 mM EGTA and no added Ca^{2+}. Responses to PMA and thrombin (with or without PMA) were measured under the same conditions. In

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: $[Ca^{2+}]_i$, cytoplasmic free Ca^{2+} concentration; PMA, 4β-phorbol 12-myristate 13-acetate; $PDec_2$, 4α-phorbol 12,13-didecanoate; $acyl_2Gro$, 1,2-diacylglycerol; PtdOH, phosphatidic acid; PtdIns-4-*P*, phosphatidylinositol 4-phosphate; PtdIns-4,5-P_2, phosphatidylinositol 4,5-bisphosphate; Ins-1,4,5-P_3, *myo*-inositol 1,4,5-trisphosphate; PAF-acether, 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphocholine.

several experiments trypsin and PAF-acether were also used as agonists. $[Ca^{2+}]_i$ was calculated from the quin-2 fluorescence measurements by the calibration procedure described by Tsien *et al.* (18) and Hallam *et al.* (2) in which the cells were lysed with 50 μM digitonin and fluorescence was determined in the presence of 1 mM Ca^{2+} (F_{max}) and at "zero" Ca^{2+} (F_{min}) by adding 4 mM EGTA at pH 8.3. $[Ca^{2+}]_i$ was calculated from the equation (18): $[Ca^{2+}]_i = K_d(F - F_{min})/(F_{max} - F)$, using a K_d of 115 nM (18). Corrections for autofluorescence and quin-2 in the medium were negligible. Secretion of dense granule constituents was monitored simultaneously with quin-2 fluorescence by measuring the release of Ca^{2+} into the medium (when extracellular Ca^{2+} = 35–50 μM) using a Radiometer Ca^{2+}-selective electrode placed in the fluorometer cuvette (12, 15).

PtdIns-4,5-[^{32}P]P_2 and [^{32}P]PtdOH were measured in platelets that were preloaded with [^{32}P]orthophosphate (19) and quin-2. The platelets were washed (12, 15) and resuspended in buffer B (without Ca^{2+}) or buffer C (with Ca^{2+}) for stimulation by thrombin. Under the conditions of our experiments the intracellular quin-2 concentration was determined (18) to be 0.6–1.0 mM, which had no substantial effect on lipid metabolism (unpublished). Lipid extracts were prepared according to Billah and Lapetina (20) and the phospholipids were separated by thin-layer chromatography (4) with a solvent system consisting of chloroform/methanol/20% aqueous methylamine, 60:36:10 (vol/vol). Radioactive lipid bands were located by autoradiography and quantitated by liquid scintillation spectroscopy. Most experiments were carried out at 23°C, but all effects described in this paper were verified at 37°C as well.

Human thrombin (2000 units/mg) was obtained from United States Biochemical (Cleveland, OH), PAF-acether, trypsin, PMA, 4α-phorbol 12,13-didecanoate ($PDec_2$), and bovine serum albumin were from Sigma, A23187 and quin-2/acetoxymethyl ester were from Calbiochem, and digitonin was from Pfaltz & Bauer (Stamford, CT). All other chemicals were of analytical grade from various sources.

RESULTS

In contrast to thrombin, PMA induces secretion after a longer lag period (1–1.5 min), at a much lower rate, to a lesser extent, and without a detectable rise of $[Ca^{2+}]_i$ (Fig. 1, traces a and f). When PMA was added immediately prior to thrombin, the rate of secretion increased 40–50%, which is consistent with earlier reports of the synergism between Ca^{2+} (mobilized by ionophores) and synthetic acyl$_2$Gro or phorbol diesters (7, 11). The positive cooperative interaction between PMA and thrombin occurred over a brief time span, after which an inhibitory action, attributable to PMA, became dominant. To investigate this unexpected inhibitory effect we incubated platelets for various times with PMA and then challenged with sufficient thrombin that normally produces a maximal rate and extent of rise of $[Ca^{2+}]_i$ and secretion. Pretreatment with PMA (10–20 ng/ml) reduced the mobilization of Ca_i^{2+} by thrombin within 30 sec; inhibition was half-maximal at 1 min and complete (>90%) in 8–9 min at 23°C (Figs. 1 and 2). However, the effect of PMA on secretion was biphasic. Despite the early reduction of thrombin-induced Ca_i^{2+} mobilization by PMA, the rate of secretion elicited by thrombin during the first minute of exposure to PMA was increased (Fig. 1, trace b; Fig. 2*A*). After 1 min, when PMA had reduced the rise of $[Ca^{2+}]_i$ by about 60% or more, the rate and extent of secretion were progressively depressed, and the lag time

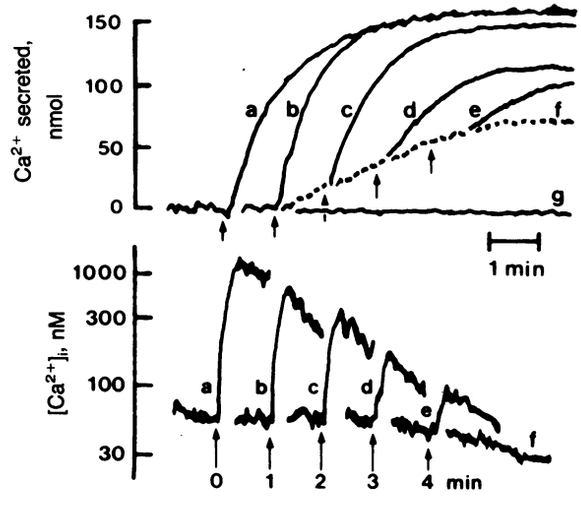

FIG. 1. Inhibition of thrombin-stimulated increase in $[Ca^{2+}]_i$ and Ca^{2+} secretion by PMA. (*Upper*) Ca^{2+} secretion. (*Lower*) $[Ca^{2+}]_i$. Trace a, control responses to thrombin (0.5 unit/ml); traces b–e, responses to thrombin added after preincubation with 20 ng of PMA per ml for 1, 2, 3, and 4 min, superimposed on the time course of secretion caused by PMA alone (trace f). Trace g, baseline extracellular Ca^{2+} = 40 μM.

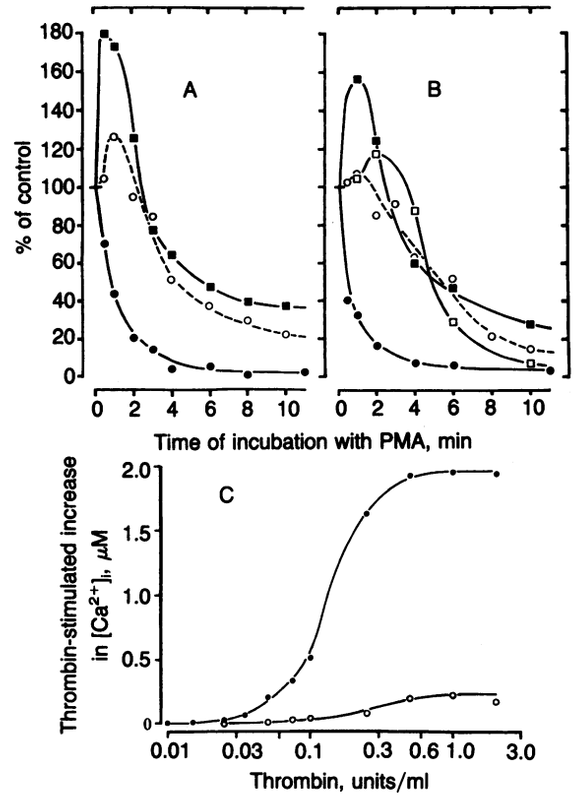

FIG. 2. Time course of PMA-induced effects on increase of $[Ca^{2+}]_i$ (●), rate of secretion (○), [^{32}P]PtdOH production (■), and breakdown of PtdIns-4,5-[^{32}P]P_2 (□). Platelets were incubated at 23°C for the indicated times with 20 ng of PMA per ml prior to stimulation with thrombin at either 0.5 unit/ml (*A*) or 2.0 units/ml (*B*). Results are expressed as % of control response to thrombin (without PMA). Ca^{2+} secreted = amount Ca^{2+} released into medium in 30 sec after the start of secretion. For evaluation of the effect of PMA on secretion the responses to thrombin (after PMA) were compared to the control response to thrombin *starting* at the equivalent level of secretion caused by PMA at each time point. [^{32}P]PtdOH was measured at 60 sec (*A*) or 30 sec (*B*) after thrombin. PtdIns-4,5-[^{32}P]P_2 was measured 30 sec after thrombin (*B*), the time at which its decrease due to thrombin is normally maximal at 23°C. (*C*) Dose–response for thrombin-induced increase of $[Ca^{2+}]_i$ in the absence (●) or presence (○) of 20 ng of PMA per ml for 3 min. Extracellular Ca^{2+} = 1 mM.

from addition of thrombin to the onset of secretion was extended. PMA inhibited the rise of $[Ca^{2+}]_i$ irrespective of temperature (23°C or 37°C) and in the presence or absence of extracellular Ca^{2+}, indicating that PMA can inhibit mobilization of intracellular Ca^{2+} stores. The inhibition of Ca_i^{2+} mobilization by PMA was not surmountable by addition of excess thrombin (Fig. 2*C*). Ca_i^{2+} mobilization by the agonists trypsin and PAF-acether was similarly inhibited (not shown), suggesting that PMA exerts a general depression of receptor-mediated mechanisms responsible for the mobilization of Ca^{2+}. In contrast to its inhibition of receptor-mediated responses, PMA had little or no influence on the increase of $[Ca^{2+}]_i$ (Fig. 3) or secretion (not shown) induced by the ionophore A23187. The phorbol diester $PDec_2$, which is inactive on protein kinase C (7) and as a tumor promoter, did not inhibit thrombin-stimulated Ca^{2+} mobilization or secretion even after incubation with platelets for 11 min at a high concentration of 100 ng/ml (Fig. 3). Ca_i^{2+} mobilization can be suppressed by stimulators of adenylate cyclase such as forskolin and prostaglandins I_2 and D_2 (12–15), but the effect of these agents is prevented by epinephrine (15), which acts through α_2-adrenergic receptors to inhibit the enzyme. However, epinephrine did not antagonize PMA (not shown), indicating that the effects of phorbol diester are unlikely to be mediated by cyclic AMP.

Because the metabolism of inositol lipids has been closely associated with changes in $[Ca^{2+}]_i$, we investigated the effects of PMA on the breakdown of PtdIns-4,5-[^{32}P]P_2 and on the formation of [^{32}P]PtdOH in platelets preloaded with both [^{32}P]phosphate and quin-2. PMA, by itself, increases the steady-state levels of PtdIns-4,5-P_2 (1.5-fold) and phosphatidylinositol 4-phosphate (PtdIns-4-*P*) (2.5-fold), at the expense of phosphatidylinositol (19, 21), but does not directly stimulate breakdown of PtdIns-4,5-P_2 or cause the formation of PtdOH. Thrombin, on the other hand, causes a dose-dependent fall in PtdIns-4,5-P_2 of 30–40% (3, 22) and a manyfold increase in PtdOH (20). When platelets pretreated for 1–2 min with 10–20 ng of PMA per ml were stimulated with 0.5 unit of thrombin per ml, the increase of [^{32}P]PtdOH was enhanced by 70–80% at a time (30–60 sec) when the rise of $[Ca^{2+}]_i$ was depressed by 50–60% (Fig. 2*A*). Thereafter (1–10 min), [^{32}P]PtdOH formation was inhibited by PMA. PtdIns-4,5-[^{32}P]P_2 was not measured in these experiments because

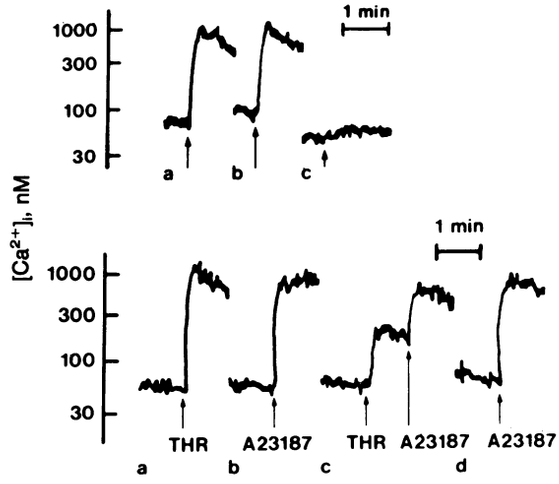

FIG. 3. Ca_i^{2+} mobilization in quin-2-loaded platelets. (*Upper*) Responses to 0.5 unit of thrombin (THR) per ml (trace a), thrombin 11 min after 100 ng of $PDec_2$ per ml (trace b), and thrombin 11 min after 10 ng of PMA per ml (trace c). (*Lower*) Responses to thrombin (0.5 unit/ml) (trace a); A23187 (30 nM) (trace b); thrombin 6 min after 10 ng of PMA per ml, followed by addition of A23187 (trace c); and A23187 6 min after PMA (trace d).

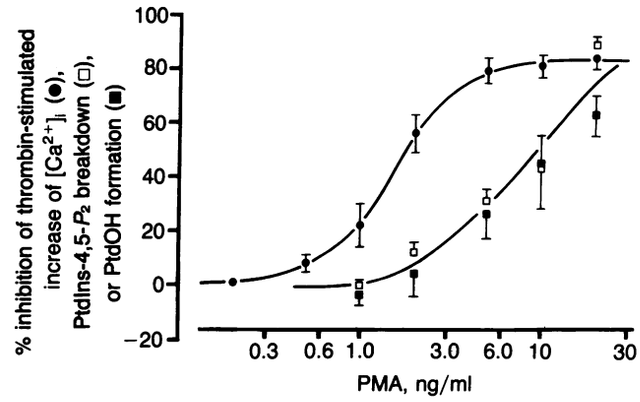

FIG. 4. Inhibition of thrombin-induced Ca_i^{2+} mobilization and lipid metabolism by PMA. Platelets preloaded with quin-2 and [^{32}P]phosphate were preincubated with the indicated concentrations of PMA for 6 min at 23°C and then stimulated with 2 units of thrombin per ml for 30 sec to measure $[Ca^{2+}]_i$ (●), PtdIns-4,5-P_2 (□), and PtdOH (■). Control thrombin-induced decrease of PtdIns-4,5-[^{32}P]P_2 was 11,714 cpm per 10^8 platelets (i.e., decreased from 39,528 ± 4150 to 27,814 ± 2890 cpm per 10^8 platelets). Control [^{32}P]PtdOH formation was increased 7.53-fold from 1102 ± 60 to 8298 ± 740 cpm per 10^8 platelets. Results are means ± SEM for triplicate measurements from three different platelet batches.

the magnitude of its breakdown at 0.5 unit of thrombin per ml was not substantial enough to accurately assess the extent of inhibition by PMA. At a higher thrombin concentration (2 units/ml), greater PtdIns-4,5-[^{32}P]P_2 breakdown and [^{32}P]PtdOH formation occurred. Pretreatment with PMA for 1–2 min caused a 10–20% greater fall of PtdIns-4,5-[^{32}P]P_2 by thrombin and a 50–60% greater formation of [^{32}P]PtdOH (Fig. 2*B*). At the same time (2 min), PMA decreased the rise of $[Ca^{2+}]_i$ by 70–80%, before any reduction in either PtdIns-4,5-[^{32}P]P_2 breakdown or [^{32}P]PtdOH production was observed (Fig. 2*B*). After longer times of preincubation (>2 min), PMA progressively inhibited both the thrombin-induced fall of PtdIns-4,5-[^{32}P]P_2 and the rise of [^{32}P]PtdOH. The inhibition of lipid metabolism by PMA was not the result of deficient Ca_i^{2+} mobilization, because it could not be reversed by raising $[Ca^{2+}]_i$ with A23187 (not shown).

The dose–response curve for inhibition of Ca_i^{2+} mobilization by PMA is shown in Fig. 4. The IC_{50} was the same (PMA IC_{50} = 2 ng/ml, 3.4 nM) at either 0.5 (not shown) or 2.0 units of thrombin per ml (Fig. 4) and encompassed the same range of PMA concentrations that activate protein kinase C in intact platelets, as determined by measurement of the phosphorylation of the M_r 40,000 protein (11). However, lipid metabolism stimulated by 2.0 units of thrombin per ml appeared less sensitive to inhibition by PMA; IC_{50} = 10 ng/ml (Fig. 4).

DISCUSSION

Tumor-promoting phorbol diesters produce an activation of platelet aggregation and secretion (23). These responses, like those in other cells, have been attributed to the activation of protein kinase C because (*i*) tumor-promoting phorbol diesters stimulate secretion by platelets at the same concentrations that activate platelet protein kinase C (7); (*ii*) phorbol diesters that do not activate protein kinase C lack the ability to stimulate platelets (7); (*iii*) inhibitors of protein kinase C prevent platelet stimulation by phorbol diesters (24); and (*iv*) secretion induced by phorbol diesters correlates well with the phosphorylation of a cytosolic protein that is a known substrate for protein kinase C in intact platelets (25). Our present results describe an additional, different, and novel action of phorbol diesters in platelets that may involve protein kinase C—i.e., the time- and dose-dependent sup-

pression of receptor-linked reactions that are associated with platelet activation. The most proximal steps thought to be involved in the transduction of receptor-mediated responses are the breakdown of PtdIns-4,5-P_2 and the rise in $[Ca^{2+}]_i$. Our experiments show that PMA, over the same range of concentrations that activate protein kinase C, produced a time-dependent suppression of Ca_i^{2+} mobilization, PtdIns-4,5-P_2 breakdown, and PtdOH formation induced by thrombin. Simultaneously, PMA also influenced thrombin-induced secretion. Initially secretion was facilitated, probably due to the previously described (11) synergism between elevated $[Ca^{2+}]_i$ and the protein kinase C pathway. However, within a short time, PMA suppressed thrombin-induced secretion with a time course that paralleled the inhibition of Ca^{2+} mobilization and lipid metabolism. Thus, despite persistent activation of protein kinase C by PMA, as indicated by phosphorylation of the M_r 47,000 protein (11), secretion due to thrombin was inhibited when the ability of thrombin to stimulate Ca_i^{2+} mobilization, PtdIns-4,5-P_2 breakdown, and PtdOH formation was eventually antagonized by PMA. Although the formation of Ins-1,4,5-P_3 by hydrolysis of PtdIns-4,5-P_2 has been proposed to be a proximal cause of Ca_i^{2+} mobilization in platelets (10), and PMA may inhibit this reaction (26), PMA substantially antagonized the thrombin-induced rise of $[Ca^{2+}]_i$ prior to any detectable inhibition of PtdIns-4,5-P_2 breakdown. This suggests that PMA may directly interfere with the stimulus-induced rise of $[Ca^{2+}]_i$ by a mechanism quite apart from its suppression of PtdIns-4,5-P_2 hydrolysis. The occurrence of these actions of PMA subsequent to the activation of protein kinase C suggests that the enzyme, directly or indirectly, could mediate reactions concerned with negative feedback upon platelet stimulatory pathways. This could conceivably ensue after receptor-mediated platelet stimulation, as a result of the increased formation of the physiological activator of protein kinase C, $acyl_2Gro$. Such negative feedback may occur in other cells as well, since PMA inhibits Ca^{2+} mobilization induced by chemotactic factors in neutrophils (27–29).

Several possible sites of action for the inhibitory effects of phorbol diester can be proposed. One possibility is that PMA causes inactivation of thrombin receptors by stimulating their phosphorylation. There is precedent for this mechanism since several types of receptors are phosphorylated by protein kinase C (30–32), and in at least one case receptor phosphorylation was correlated with decreased function (32). However, the ability of PMA to suppress Ca_i^{2+} mobilization in platelets more effectively than PtdIns-4,5-P_2 or PtdOH metabolism prompts the view that PMA has some postreceptor site of action, such as on receptor-associated coupling factors (i.e., GTP-binding proteins), which may be involved in regulating phosphatidylinositol phosphate metabolism (33, 34) and/or the process of intracellular Ca_i^{2+} mobilization (35). PMA may also suppress the rise of $[Ca^{2+}]_i$ by stimulating Ca^{2+} transport (28). The available evidence is insufficient to come to any definite conclusions about these various possible sites of action of PMA. However, further understanding of the mechanism of action of PMA on phosphatidylinositol phosphate metabolism and Ca^{2+} mobilization, and its relationship to protein kinase C, will provide considerable insight into the mechanisms for transmembrane signal transduction.

This work was supported by National Institutes of Health Grants HL18937 and AI13734.

1. Rink, T. J., Smith, S. W. & Tsien, R. Y. (1982) *FEBS Lett.* **148,** 21–26.
2. Hallam, T. J., Sanchez, A. & Rink, T. J. (1984) *Biochem. J.* **218,** 819–827.
3. Vickers, J. D., Kinlough-Rathbone, R. L. & Mustard, J. F. (1984) *Biochem. J.* **219,** 25–31.
4. Shukla, S. D. & Hanahan, D. J. (1982) *Biochem. Biophys. Res. Commun.* **106,** 697–703.
5. Downes, C. P. & Michell, R. H. (1982) *Cell Calcium* **3,** 467–502.
6. Kishimoto, A., Takai, Y., Mori, T., Kikkawa, U. & Nishizuka, Y. (1980) *J. Biol. Chem.* **255,** 2273–2276.
7. Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U. & Nishizuka, Y. (1982) *J. Biol. Chem.* **257,** 7847–7851.
8. Streb, H., Irvine, R. F., Berridge, M. J. & Schulz, I. (1983) *Nature (London)* **306,** 67–69.
9. Burgess, G. M., Godfrey, P. P., McKinney, J. S., Berridge, M. J., Irvine, R. F. & Putney, J. W. (1984) *Nature (London)* **309,** 63–66.
10. O'Rourke, F., Halenda, S. P., Zavoico, G. B. & Feinstein, M. B. (1985) *J. Biol. Chem.* **260,** 956–962.
11. Yamanishi, J., Takai, Y., Kaibuchi, K., Sano, K., Castagna, M. & Nishizuka, Y. (1983) *Biochem. Biophys. Res. Commun.* **112,** 778–786.
12. Feinstein, M. B., Egan, J. J., Sha'afi, R. I. & White, J. (1983) *Biochem. Biophys. Res. Commun.* **113,** 598–604.
13. Rink, T. J. & Smith, S. W. (1983) *J. Physiol.* **338,** 66–67P.
14. Yamanishi, J., Kawahara, Y. & Fukuzaki, H. (1983) *Thrombosis Res.* **32,** 183–188.
15. Zavoico, G. B. & Feinstein, M. B. (1984) *Biochem. Biophys. Res. Commun.* **120,** 579–585.
16. Zavoico, G. B., Halenda, S. P., Chester, D. W. & Feinstein, M. B. (1985) in *Prostaglandins, Leukotrienes and Lipoxins*, ed. Bailey, J. (Plenum, New York), in press.
17. Watson, S. P., McConnell, R. T. & Lapetina, E. G. (1984) *J. Biol. Chem.* **259,** 13199–13203.
18. Tsien, R. Y., Pozzan, T. & Rink, T. J. (1982) *J. Cell Biol.* **94,** 325–334.
19. Halenda, S. P. & Feinstein, M. B. (1984) *Biochem. Biophys. Res. Commun.* **124,** 507–513.
20. Billah, M. M. & Lapetina, E. G. (1982) *J. Biol. Chem.* **257,** 12705–12708.
21. De Chaffoy de Courcelles, D., Roevens, P. & van Belle, H. (1984) *FEBS Lett.* **173,** 389–393.
22. Broekman, M. J. (1984) *Biochem. Biophys. Res. Commun.* **120,** 226–231.
23. Zucker, M. B., Troll, W. & Belman, S. (1974) *J. Cell Biol.* **60,** 325–336.
24. Sanchez, A., Hallam, T. J. & Rink, T. J. (1983) *FEBS Lett.* **164,** 43–46.
25. Imaoka, T., Lynham, J. A. & Haslam, R. J. (1983) *J. Biol. Chem.* **258,** 11404–11414.
26. Labarca, R., Janowsky, A., Patel, J. & Paul, S. M. (1984) *Biochem. Biophys. Res. Commun.* **123,** 703–709.
27. Schell-Frederick, E. (1984) *Cell Calcium* **5,** 237–251.
28. Lagast, H., Pozzan, T., Waldnogel, F. A. & Lew, P. D. (1984) *J. Clin. Invest.* **73,** 878–883.
29. Naccache, P. H., Molski, T. F. P., Borgeat, P., White, J. R. & Sha'afi, R. I. (1985) *J. Biol. Chem.* **260,** 2125–2131.
30. Jacobs, S., Sahyoun, N. E., Saltiel, A. R. & Cuatrecasas, P. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6211–6213.
31. Iwashita, S. & Fox, C. F. (1984) *J. Biol. Chem.* **259,** 2559–2567.
32. Cochet, C., Gill, G. N., Meisenhelder, J., Cooper, J. A. & Hunter, T. (1984) *J. Biol. Chem.* **259,** 2553–2558.
33. Haslam, R. J. & Davidson, M. M. L. (1984) *Biochem. J.* **222,** 351–361.
34. Volpi, M., Naccache, P. H., Molski, T. F. P., Shefcyk, J., Huang, C.-K., Marsh, M. L., Munoz, J., Becker, E. L. & Sha'afi, R. I. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2708–2712.
35. Molski, T. F. P., Naccache, P. H., Marsh, M. L., Kermode, J., Becker, E. L. & Sha'afi, R. I. (1984) *Biochem. Biophys. Res. Commun.* **124,** 644–650.