Arachidonic acid mobilization in platelets: The possible role of protein kinase C and G-proteins

by M. B. Feinstein and S. P. Halenda*

Department of Pharmacology, University of Connecticut Health Center, Farmington (Connecticut 06032, USA)

Summary. A major route for the release of arachidonic acid from platelet phospholipids appears to be catalyzed by a phospholipase A_2 that can be stimulated by a rise of cytosolic Ca^{2+}. This paper discusses certain other mechanisms for regulation of this process. Release of arachidonic acid by calcium ionophores is potentiated by pretreatment with stimulators of protein kinase C; e.g. diglyceride, phorbol esters and the terpene diester mezerein. This effect appears to be coincident with phosphorylation of a certain group of proteins (not 47 KDa protein), and is sensitive to depletion of ATP, activation of Ca^{2+} dependent phosphatase, and the kinase C inhibitor H-7, but is unaffected by Na^+/H^+ exchange inhibitors. Recent results in other cell types strongly indicate that phospholipase A_2 is also directly under control of certain GTP-binding proteins.
Key words. Platelets; calcium; phospholipase A_2; G-proteins; arachidonic acid.

Arachidonic acid release from platelet phospholipids is known to occur through two major routes: (a) hydrolysis by DG-lipases of stearylarachidonyl-diacylglycerol which arises from hydrolysis of phosphatidylinositol 4,5-biphosphate, and (b) hydrolysis of phospholipids at the fatty acyl 2-position of mainly PC† and PE by phospholipase A_2. The second pathway appears to be the major source of arachidonic acid since the mass of released arachidonic acid from PC and PE greatly exceeds the amount available in the pool of phosphoinositides [3, 37]. It is widely believed that changes in free Ca^{2+} levels of the cytosol play an important role in the activation of cellular PLA_2. However, with few exceptions, the amount of Ca^{2+} needed to activate membrane PLA_2's is usually far above the physiological range. One possible explanation for these findings is that some other factors modulate the Ca^{2+} sensitivity of the enzyme. Synergistic interactions between protein kinase C and Ca^{2+} are well known [28] and suggest the possibility that a similar synergism might extend to regulation of arachidonic acid release from phospholipids by PLA_2. Indeed, a synergism between agents that stimulate C kinase activity and Ca^{2+} promoting the release of arachidonic acid has been demonstrated in platelets [15, 27], chromaffin cells [12] and neutrophils [26, 41]. In this paper we review the possible mechanisms that may underlie this effect, and consider its potential physiological relevance.

The requirement for Ca^{2+} in the activation of arachidonic acid release

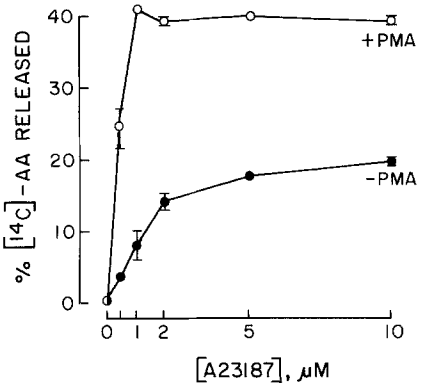
It is generally assumed that a sufficient increase of cytosolic free Ca^{2+} is responsible for the activation of phospholipase A_2, but direct support for this hypothesis is lacking, most notably because PLA_2 activities identified have usually required Ca^{2+} concentrations greatly in excess of the levels actually attained in intact stimulated platelets. Recently, Loeb and Gross [24] identified and purified a sheep platelet PLA_2 that was essentially fully activated at 800 nM Ca^{2+}. They also provided evidence for various isoforms of the enzyme with differing substrate specificities. Further evidence that a rise of cytosolic Ca^{2+} may be necessary, and sufficient, to activate PLA_2 comes from experiments demonstrating the release of arachidonic acid from phospholipids (PC, PE and PI) by Ca^{2+} ionophores. These conditions precluded activation of the phospholipase C pathway, so that little or no fatty acid was derived from diacylglycerol [34]. Further proof that Ca^{2+} can be directly responsible for arachidonic acid release in intact platelets was provided by Halenda and Rehm [13]. They depleted about 90% of platelet Ca^{2+} by incubation with A 23187 plus EGTA. Subsequently, ionophore was removed and the platelet lipids were labeled with radioactive arachidonic acid and then made selectively permeable to Ca^{2+} with ionophore plus Ca^{2+}/EGTA buffers. Release of arachidonic acid was produced by free Ca^{2+} concentrations in the same physiological range attained in thrombin-stimulated platelets; i.e. 0.2 to 3.0 μM Ca^{2+}.

The enhancement of Ca^{2+}-dependent arachidonic acid release by activators of protein kinase C

Despite the clear association between Ca^{2+} and arachidonic acid mobilization it is likely that other mechanisms exist to regulate this pathway, as is the case with other Ca^{2+}-dependent processes. Indeed, complex positive and negative feedback loops exist between Ca^{2+} and the diacylglycerol/Ca^{2+}/phosphatidylserine activated protein kinase C in platelets; e.g. Ca^{2+} and DG initially synergistically promote aggregation and secretion, but protein kinase C may terminate release of intracellular Ca^{2+} [25, 43] by activating a phosphatase that destroys IP_3 [7] and by inhibiting hydrolysis of PIP_2 [33, 43]. Several groups have identified a class of proteins (i.e. lipocortins) that inhibit hydrolysis of phospholipids by pancreatic PLA_2, and whose activity can be overcome by phosphorylations mediated by protein kinase C [23] and tyrosine kinases [17, 19, 30]. This work has led to the hypothesis that the lipocortins serve as natural inhibitors of PLA_2, and are themselves inactivated by tyrosine and C kinases [18]. The release of lipocortin's inhibition and the concurrent rise of Ca^{2+} presumably permits PLA_2 activity to release arachidonic acid.

With these findings in mind we undertook to study the role that protein kinase C might play in arachidonic acid metabolism in platelets. Cellular kinase C can be activated by exogenous DG analogs and tumor-promoting phorbol esters [28], and we initially studied its role by pretreating washed platelets with these agents and then stimulated them with Ca^{2+} ionophores [15]. The activators of protein kinase C produced a dose- and time-dependent enhancement of arachidonic acid release, even in aspirinized platelets in the presence of creatine kinase/creatine phosphate to prevent activation of the PLC pathway by ADP and TXA 2. At low concentrations of ionophore the release of arachidonic acid (and the production of TXB 2) was dramatically increased (e.g. about 10-fold), but also the maximum release of arachidonic acid by higher concentrations of ionophores was increased by 2-fold (fig.). Further confirmation of this effect was provided by Halenda and Rehm [13] who employed Ca^{2+}-depleted platelets and showed that the release of arachidonic acid by extracellular Ca^{2+}/EGTA buffers plus A 23187 was also enhanced by PMA. The potentiation of ionophore-induced thromboxane release in platelets by PMA was also reported by Mobley and Tai [27], and enhancement of arachidonic acid release by PMA has also been observed in neutrophils [26, 41].

Since the kinase C activators were by themselves unable to directly release arachidonic acid in unstimulated platelets the interaction with the ionophores suggested that phorbol esters increased the sensitivity of the system to Ca^{2+} and/or made available more substrate or PLA_2. Two possibilities for these actions come to mind. The first, and most obvious, is that kinase C itself directly mediated these effects, but a second plausible explanation is that the enhancing agents act



Potentiation of ionophore-induced arachidonic acid release from ^{3}H-arachidonic acid-labeled intact platelets by PMA (100 ng/ml)

on another enzyme or regulatory system, or act non-enzymatically to increase phospholipid hydrolysis [10, 31]. The involvement of protein kinase C in this response was implied by the following evidence (ref. 15, and unpublished results): (a) alpha-PDD, a phorbol ester inactive as a tumor-promoter or kinase C activator, did not promote arachidonic acid release, (b) three different types of kinase C activators were effective; i.e. OAG, phorbol esters (PMA, PDBu), and the non-phorbol ester mezerein, and (c) the effects of PMA, OAG and mezerein were partially inhibited by the kinase C inhibitor H-7. Furthermore, the PMA-enhanced release of arachidonic acid was abolished by incubation of the platelets with inhibitors (2-deoxyglucose plus antimycin A) that deplete the metabolic pool of ATP. This treatment did not inhibit release of arachidonic acid by ionophores. This result clearly implicates some energy-dependent process requiring ATP and/or GTP in the action of PMA, and further suggests that PLA_2 itself is relatively independent of such control when cytoplasmic Ca^{2+} is sufficiently elevated.

Several other mechanisms for these effects of OAG, phorbol esters and mezerein must be considered: (a) a membrane perturbing action unrelated to kinase C that promotes hydrolysis of phospholipids [10, 31]; (b) phosphorylation of some protein that regulates PLA_2 activity (e.g. lipocortins); (c) modulation of intracellular pH (pH_i) by the Na^+/H^+ exchanger which can be stimulated by protein kinase C; (d) the cytoskeleton may play a role in arachidonic acid release and PMA can selectively affect some part of the cytoskeleton; (e) involvement of a GTP-binding protein (G-protein) either directly or indirectly in the regulation of PLA_2; (f) a secretion-related event in which a released product, or insertion of the granule membranes into the plasma membrane promotes lipid hydrolysis, provides specific substrate or adds new enzyme or an enzyme activator to the plasma membrane. A secreted product has been ruled out (unpublished experiments). It should be borne in mind that the exact localization of PLA_2, in plasma and/or internal membranes, is not known with certainty. Several of these possible mechanisms are discussed below.

Involvement of the Na^+/H^+ exchanger

The Na^+/H^+ exchanger that is activated by thrombin can raise pH_i by about 0.15 units [44] and may play a role in arachidonic acid release [38]. Inhibition of Na^+/H^+ exchange by removal of extracellular Na^+ or addition of amiloride-type exchange inhibitors causes pH_i of stimulated platelets to fall rather than rise [44], as is normally the case, and these conditions suppress arachidonic acid release by weak agonists and low concentrations of thrombin [38]. This inhibitory effect was weak or absent when the thrombin concentration was above 0.1 U/ml. It has been claimed that activation of the Na^+/H^+ exchanger is a necessary *prerequisite* for mobilization of Ca^{2+} [35] which could explain the effect of the exchange-inhibitors on arachidonic acid release. Our evidence does not support this thesis, since we find that intracellular Ca^{2+} mobilization by low concentrations of thrombin is only partially inhibited, and barely affected at 1.0 U/ml despite a fall of pH_i (0.1–0.15 units) caused by thrombin in the presence of the Na^+/H^+ exchange inhibitors [44]. Also, acidification of the platelet cytosol with nigericin reduced somewhat, but did not abolish, Ca^{2+} mobilization by thrombin [44]. Further, and most significantly, the time course of cytosolic alkalinization produced by thrombin, which is due to activation of the Na^+/H^+ exchanger, follows rather than precedes Ca^{2+} mobilization [44]. Nevertheless, a fall in pH_i caused by exchange-blockers or absence of Na^+ could reduce PLA_2 activity (which normally has an alkaline pH optimum), and the ability of PMA to stimulate the Na^+/H^+ exchanger [36, 44] might account for its potentiation of arachidonic acid release. In fact we found that complete inhibition of the Na^+/H^+ exchanger by DMA or EIPA had no effect on the potentiation of arachidonic acid release by PMA (Halenda and Feinstein, unpublished results). Under these conditions of maximal Ca^{2+} mobilization and activation of kinase C the Na^+/H^+ exchanger appears to have no role in arachidonic acid release. However, when Ca^{2+} mobilization and activation of kinase C are marginal, as with weak agonists and low concentrations of thrombin the pH_i and Na_i^+ changes may be play a influential, but permissive, role [38].

Protein phosphorylation

The most striking substrate for platelet kinase C is a 47 KDa phosphoprotein [20]. Recently this protein has been associated with a phosphatase activity that converts IP_3 (1,4,5-inositol trisphosphate) to IP_2 (1,4-inositol bisphosphate), thereby terminating the Ca^{2+}-mobilizing activity of IP_3 [7]. We find no correspondence between the phosphorylation of the 47 KDa protein and the time course or dose-response for PMA-enhanced release of arachidonic acid (Halenda et al. [15], and unpublished results). Consideration of the possibility that the 47 KDa protein is lipocortin [8] was unfounded since the existing published data clearly identify them as very different proteins with respect to pI's [19, 20] and molecular weights; e.g. lipocortins I and II are approximately 35 to 37 KDa [19, 30], not 47 KDa.

By studying the responses of platelets at 21–23 °C we have been able to distinguish quite different rates for the phosphorylation of proteins, which we roughly classify as 'fast' and 'slow'. Phosphorylation of the 47 KDa protein is fast, being complete within 30–60 s. In contrast proteins of 65, 41, 35–36, and 21 KDa are phosphorylated much slower. The time course for the phosphorylation of these latter proteins, and the dose-response for PMA, correspond closely to the time course and dose-response for enhancement of arachidonic acid release by PMA. Furthermore, ATP-depletion as well as treatment with A 23187 just prior to PMA (which may activate Ca^{2+}-dependent protein phosphatase) severely depresses protein phosphorylation and prevents enhancement of arachidonic acid release by PMA. The slowly phosphorylated proteins have not been completely identified, but the 41 KDa protein migrates by 2-dimensional gel electrophoresis identically with a 41 KDa protein ADP-ribosylated by pertussis toxin, and the 35–36 KDa protein has a pI similar to that reported for lipocortin [19].

The possible role of lipocortins

It has been proposed that proteins termed lipocortins (p35, p36 and lipocortin 85 – an oligomer of p36 and two

10 KDa subunits) are endogenous inhibitors of PLA_2 [11, 18, 19, 23, 30, 40]. That activity of lipocortins I (p35) and II (p36) appears to be lost when the proteins are phosphorylated by tyrosine kinase and/or kinase C [17]. However, recent findings cast doubt on this hypothesis, since the inhibitory activity of lung calpactins (believed identical to lipocortins) was only apparent at very low substrate concentration and was abolished when phospholipid concentration reached 200 μM despite a calpactin/PLA_2 ratio of 10:1 [9]. Furthermore, no complex formation between pancreatic PLA_2 and calpactins at a 100-fold molar excess was observed. Because the calpactins bind to phospholipid in the presence of Ca^{2+} it was suggested that the proteins prevented access of PLA_2 to substrate, and that the inhibitory action of lipocortins on arachidonic acid release by pancreatic PLA_2 was attributable to the very low concentrations of substrate (arachidonic acid-labeled *E. coli* membranes) used in most previous studies. The true role, if any, of lipocortins (calpactins) in the regulation of arachidonic acid release in cells has yet to be fully explored since their activity has not been assessed on relevant cellular PLA_2's whose Ca^{2+} dependency and substrate specificities may be quite different than the soluble pancreatic PLA_2 commonly used to assess lipocortin activity. Nevertheless, the case for regulation of PLA_2 by phosphorylation of lipocortins currently remains in doubt.

The possible role of G-proteins

The most exciting new developments in the regulation of arachidonic acid release concern the involvement of GTP-binding proteins. Agonist-induced release of arachidonic acid in mast cells, neutrophils and fibroblasts is inhibited by pertussis toxin, but this effect may or may not be selective since the G-protein-dependent phosphoinositide hydrolysis pathway is also blocked. The effect of the toxin on arachidonic acid release in these cells may be indirect since DG formed from the latter pathway is a substrate for lipases that release arachidonic acid, and DG may also affect arachidonic acid through activation of kinase C or PLA_2. However, in FRTL 5 rat thyroid cells Burch et al. [4] showed that activation of phospholipase C and arachidonic acid release caused by α_1-adrenergic receptors were probably mediated by different G-proteins. Although both effects were produced by GTPγS only the arachidonic acid release was blocked by pertussis toxin, indicating the involvement of different G-proteins for each pathway. The most direct evidence for regulation of PLA_2 by a G-protein was obtained by Jelsema et al. [21], who found that purified $\beta\gamma$ subunits of transducin could stimulate phospolipase A_2 activity of membranes of photoreceptors and other cells. The free α subunit was much less active and may inhibit $\beta\gamma$. These data suggest that dissociation of G-protein could activate PLA_2, whereas recombination of free α with $\beta\gamma$ to form the $\alpha\beta\gamma$ heterotrimer could turn off PLA_2 activation. In platelets, thrombin causes rapid reversible dissociation of G-protein oligomers that is blocked by cAMP [14]. Since PMA also appears to affect the state of association of G-protein oligomers [14], and the function of alpha subunits [22], it is possible that these effects of PMA influence the release of arachidonic acid.

At least two categories of G-proteins have been identified in cells; those that are ADP-ribosylated by bacterial toxins (e.g. Gi and Gs) and those that bind GTP, but are not ADP-ribosylated by toxins, such as the putative phospholipase C-activator Gp and the $p21^{N-ras}$ protein [42]. In platelets, proteins of the first type have been implicated in PIP_2 hydrolysis by phospholipase C [2, 16] and in Ca^{2+} mobilization since both responses are GTP-dependent and blocked by pertussis toxin [2, 29]. Two ADP-ribosylated proteins of 41 and 39 KDa were identified as substrates for the toxin in the latter study. In platelets, certain agonists (e.g. thrombin) cause receptor-induced dissociation of G-protein oligomers (ADP-ribosylatable by pertussis toxin), resulting in an increase of free α (GTPase) and $\beta\gamma$ subunits that transduce various metabolic reactions [29]. This may be a key early step in stimulus-response coupling, leading to the hydrolysis of PIP_2 to form the intracellular messengers IP_3 and DG, and ultimately the Ca^{2+}-dependent release of arachidonic acid. G-proteins of the second type (i.e. non-toxin sensitive), such as $p21^{N-ras}$, may also regulate PLC [42], and could be important in platelet physiology. Bhullar and Haslam [1] have identified a new major GTP-binding protein of 27 KDa in platelet membranes, along with minor GTP-binding proteins of 25, 24 and 23 KDa. This important new finding indicates that regulation of metabolic processes by GTP-binding proteins in platelets is surely much more complex than is our current understanding. GTP-dependent activation of membrane phospholipase C, and Ca^{2+} mobilization in permeabilized platelets [2], and in an in vitro system consisting of platelet plasma membranes plus IP_3-sensitive Ca^{2+} accumulating vesicles [29], have been shown to be sensitive to blockade by pertussis toxin. It remains to be shown whether the PLA_2 pathway for release of arachidonic acid in platelets is mediated by specific receptors and/or specific G-proteins, and whether these G-proteins are toxin-sensitive or toxin-insensitive.

† Abbreviations: PLA_2, phospholipase A_2; PLC, phospholipase C; IP_3, inositol 1,4,5-trisphosphate; PIP_2 phosphatidylinositol 4,5-bisphosphate; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol.

Acknowledgment. This study was supported by NIH Grant HL 18937.

* Present address: Dept. of Pharmacology, University of Missouri, Columbia, Missouri.

1 Bhullar, R. P., and Haslam, R. J., New GTP-binding proteins in platelet and other cell membranes. Fedn Proc. *46* (1987) 422.
2 Brass, L. F., Laposta, M., Banga, H. S., and Rittenhouse, S. E., Regulation of the phosphoinositide hydrolysis pathway in thrombin-stimulated platelets by a pertussis toxin-sensitive guanine nucleotide-binding protein. J. biol. Chem. *261* (1986) 16838–16847.
3 Broekman, M. J., Stimulated platelets release equivalent amounts of arachidonate from phosphatidylcholine, phosphatidylethanolamine and inositides. J. Lipid Res. *27* (1986) 884–891.
4 Burch, R. M., Luini, A., and Axelrod, J., Phospholipase A_2 and phospholipase C are activated by distinct GTP-binding proteins in response to α_1-adrenergic stimulation in FRTL5 thyroid cells. Proc. natl Acad Sci. USA *83* (1986) 7201–7205.
5 Burch, R. M., and Axelrod, J., A GTP-binding protein (G-protein) regulates phospholipase A_2 in RAW 264.7 macrophages. Fedn Proc. *46* (1987) 703.
6 Chiarugi, V., Pasquali, F., Vannuchi, S., and Ruggiero, M., Point mutated $p21^{ras}$ couples muscarinic receptor to calcium channels and polyphosphoinositide hydrolysis. Biochem. biophys. Res. Commun. *141* (1986) 591–599.
7 Connolly, T. M., Lawing, W. J. Jr, and Majerus, P. W., Protein kinase C phosphorylates human platelet inositol trisphosphate 5'-phosphomonoesterase, increasing the phosphatase activity. Cell *46* (1986) 951–958.
8 Crouch, M. F., and Lapetina, E. G., Phosphorylation of the 47 KDa protein in g-thrombin stimulated human platelets does not activate phospholipase A_2: evidence against lipocortin. Biochem. biophys. Res. Commun. *141* (1986) 459–465.
9 Davidson, F. F., Dennis, E. A., Powell, M., and Glenney, J. R. Jr, Inhibition of phospholipase A_2 by 'lipocortins' and calpactins; an effect of binding to substrate phospholipids. J. biol. Chem. *262* (1987) 1698–1705.
10 Dawson, R. M. C., Irvine, R. F., Bray, J., and Quinn, P. J., Long-chain unsaturated diacylglycerols cause a perturbation in the structure of phospholipid bilayers rendering them susceptible to phospholipase attack. Biochem. biophys. Res. Commun. *125* (1984) 836–842.

11 Flower, R. J., Wood, J. N., and Parente, L., Macrocortin and the mechanism of action of the glucocorticoids. Adv. Inflamm. Res. *7* (1984) 61–69.
12 Frye, R. A., and Holz, R. W., Arachidonic acid release and catecholamine secretion from digitonin-treated chromaffin cells: effects of micromolar calcium, phorbol ester and protein alkylating agents. J. Neurochem. *44* (1985) 265–273.
13 Halenda, S. P., and Rehm, A. G., Direct stimulation of arachidonic acid release by calcium in human platelets. Fedn Proc. *46* (1987) 398.
14 Halenda, S. P., Volpi, M., Zavoico, G. B., Sha'afi, R. I., and Feinstein, M. B., Effects of thrombin, phorbol myristate acetate and prostaglandin D2 on 40–41 KDa protein that is ADP ribosylated by pertussis toxin in platelets. FEBS Lett. *204* (1986) 341–346.
15 Halenda, S. P., Zavoico, G. B., and Feinstein, M. B., Phorbol esters and oleoyl acetoyl glycerol enhance release of arachidonic acid in platelets stimulated by Ca^{2+} ionophore A 23187. J. biol. Chem. *260* (1985) 12484–12491.
16 Haslam, R. J., and Davidson, M. M. L., Receptor-induced diacylglycerol formation in permeabilized platelets. J. Recep. Res. *4* (1984) 605–629.
17 Hirata, F., Matsuda, K., Notsu, Y., Hattori, T., and Del Carmine, R., Phosphorylation at a tyrosine residue of lipomodulin in mitogen-stimulated murine thymocytes. Proc. natl Acad. Sci. USA *81* (1984) 4717–4721.
18 Hirata, F., Roles of lipomodulin: a phospholipase inhibitory protein in immunoregulation. Adv. Inflamm. Res. *7* (1984) 71–78.
19 Huang, K-S., Wallner, B. P., Mattaliano, R. J., Tizard, R., et al., Two human 35 kd inhibitors of phospholipase A_2 are related to substrates of pp60v-src and the epidermal growth factor receptor/kinase. Cell *46* (1986) 191–199.
20 Imaoka, T., Lynham, J. A., and Haslam, R. J., Purification and characterization of the 47,000-dalton protein phosphorylated during degranulation of human platelets. J. biol. Chem. *258* (1983) 11404–11414.
21 Jelsema, C. L., Burch, R. M., and Axelrod, J., The ag subunits of the GTP-binding protein activate and the α subunit inhibits phospholipase A_2. J. Cell Biol. *103* (1986) 456a.
22 Katada, T., Gilman, A. G., Watanabe, Y., Bauer, S. J., and Jakobs, K. H., Protein kinase C phosphorylates the inhibitory guanine nucleotide binding regulatory component and apparently suppresses its function in hormonal inhibition of adenylate cyclase. Eur. J. Biochem. *151* (1985) 431–437.
23 Khanna, N. C., Tokuda, M., and Waisman, D. M., Phosphorylation of lipocortins in vitro by protein kinase C. Biochem. biophys. Res. Commun. *141* (1986) 547–554.
24 Loeb, L. A., and Gross, R. W., Identification and purification of sheep platelet phospholipase A_2 isoforms. J. biol. Chem. *261* (1986) 10467–10470.
25 MacIntyre, D. E., McNicol, A., and Drummond, A. H., Tumor promoting phorbol esters inhibit agonist-induced phosphatidate formation and Ca^{2+} flux in human platelets. FEBS Lett. *180* (1985) 160–164.
26 McColl, S. R., Hurst, N. P., and Cleland, L. G., Modulation by phorbol myristate of arachidonic acid release and leukotriene synthesis by human polymorphonuclear leukocytes stimulated with A 23187. Biochem. biophys. Res. Commun. *141* (1986) 399–404.
27 Mobley, A., and Tai, H. H., Synergistic stimulation of thromboxane biosynthesis by calcium ionophore and phorbol ester or thrombin in human platelets. Biochem. biophys. Res. Commun. *130* (1985) 717–723.
28 Nishizuka, Y., The role of protein kinase C in cell surface signal transduction and tumor promotion. Nature *308* (1984) 693–698.
29 O'Rourke, F., Zavoico, G. B., Smith, L. H. Jr, and Feinstein, M. B., Stimulus-response coupling in a cell-free platelet membrane system: GTP-dependent release of Ca^{2+} by thrombin, and inhibition by pertussis toxin and monoclonal antibody that blocks calcium release by IP_3. FEBS Lett. *214* (1987) 176–181.
30 Pepinsky, R. B., and Sinclair, L. K., Epidermal growth factor-dependent phosphorylation of lipocortin. Nature *321* (1986) 81–84.
31 Pollock, W. K., Rink, T. J., and Irvine, R. T., Liberation of [3H]-arachidonic acid and changes in cytosolic free calcium in fura-2 loaded human platelets stimulated by ionomycin and collagen. Biochem. J. *235* (1986) 869–877.
32 Prescott, S. M., and Majerus, P. W., Characterization of 1,2-diacylglycerol hydrolysis in human platelets. J. biol. Chem. *258* (1983) 764–769.
33 Rittenhouse, S. E., and Sasson, J., Mass changes in myoinositol trisphosphate in human platelets stimulated by thrombin. Inhibitory effects of phorbol ester. J. biol. Chem. *260* (1985) 8657–8660.
34 Rittenhouse, S. E., Activation of human platelet phospholipase C by ionophore A 23187 is totally dependent upon cyclo-oxygenase products and ADP. Biochem. J. *222* (1984) 103–110.
35 Siffert, W., and Akkerman, J. W. N., Activation of sodium-proton exchange is a prerequisite for Ca^{2+} mobilization in human platelets. Nature *325* (1987) 456–458.
36 Siffert, W., and Scheid, P., A phorbol ester and 1-oleoyl-2-acetylglycerol induce Na^+/H^+ exchange in human platelets. Biochem. biophys. Res. Commun. *141* (1986) 13–19.
37 Smith, J. B., Dangelmaier, C., and Mauco, G., Measurement of arachidonic acid liberation in thrombin-stimulated human platelets. Biochim. biophys. Acta *835* (1985) 344–351.
38 Sweatt, J. D., Johnson, S. L., Cragoe, E. J. Jr, and Limbird, L. E., Inhibitors of Na^+/H^+ exchange block stimulus-provoked arachidonic acid release in human platelets. J. biol. Chem. *260* (1985) 12910–12919.
40 Touqui, L., Rothut, B., Shaw, A. M., Fradin, A., Vargaftig, B. B., and Russo-Marie, F., Platelet activation – a role for a 40 K anti-phospholipase A_2 protein indistinguishable from lipocortin. Nature, Lond. *321* (1986) 177–180.
41 Volpi, M., Molski, T. F. P., Naccache, P. H., Feinstein, M. B., and Sha'afi, R. I., Phorbol 12-myristate, 13-acetate potentiates the action of the calcium ionophore in stimulating arachidonic acid release and production of phosphatidic acid in rabbit neutrophils. Biochem. biophys. Res. Commun. *128* (1985) 594–600.
42 Wakelam, M. J. O., Davies, S. A., Houslay, M. D., McKay, I., Marshall, C. J., and Hall, A., Normal $p21^{N-ras}$ couples bombesin and other growth factor receptors to inositol phosphate production. Nature *323* (1986) 173–176.
43 Zavoico, G. B., Halenda, S. P., Sha'afi, R. I., and Feinstein, M. B., Phorbol myristate acetate inhibits thrombin-stimulated Ca^{2+} mobilization and phosphatidylinositol 4,5-bisphosphate hydrolysis in human platelets. Proc. natl Acad. Sci. USA *82* (1985) 3859–3862.
44 Zavoico, G. B., Cragoe, E. J. Jr, and Feinstein, M. B., Regulation of intracellular pH in platelets. J. biol. Chem. *261* (1986) 13160–13167.

0014-4754/88/020101-04$1.50 + 0.20/0