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# EFFECT OF PCR 4099 ON ADP-INDUCED CALCIUM MOVEMENTS AND PHOSPHATIDIC ACID PRODUCTION IN RAT PLATELETS

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**Abstract**—Antiplatelet activity of PCR 4099, an analogue of ticlopidine, resides in its specific effect against exogenous as well as released ADP. This study investigated in rat platelets the effects of the drug on ADP-induced shape change, elevation of cytosolic free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) and hydrolysis of inositol phospholipids, monitored as [$^{32}$P]phosphatidic acid formation. Shape change and influx of $Ca^{2+}$ ions across the plasma membrane were not modified after PCR 4099 administration using aspirin-treated platelets. On the other hand, phosphatidic acid formation and calcium mobilization from internal stores were strongly inhibited. These results suggest that PCR 4099 leaves intact the machinery involved in ADP-induced platelet shape change and influx of calcium ions, but inhibits an early step in the ADP–response coupling leading to inositol phospholipid hydrolysis and aggregation.

Ticlopidine and its analogue PCR 4099 inhibit *ex vivo* the aggregation of platelets in response to a variety of agents such as ADP,§ collagen, arachidonic acid, thrombin, epinephrine and calcium ionophore A23187 (reviewed in Refs 1 and 2). We have found recently that the broad spectrum anti-aggregating activity of these drugs resides in their specific effect against ADP [3].

ADP is known to be a major regulator of platelet behaviour [4–8]. Its interaction with platelets leads to shape change, exposure of fibrinogen binding sites and aggregation [9–12]. ADP induces these responses by interacting with specific cell surface receptors [13–16]. Like many other agonists, ADP produces in platelets a rapid elevation of the cytoplasmic free calcium concentration [12, 17, 18], resulting not only from an influx across the plasma membrane, but also from the discharge from internal stores. The latter is thought to involve generation of inositol-1,4,5-trisphosphate ($IP_3$) from phosphatidylinositol-4,5-bisphosphate by phospholipase C [19, 20]. However, whether ADP is able to initiate phospholipase C activation in platelets is still a matter of controversy and might depend on the animal species considered [17, 18, 21–23]. For example, studies in rat platelets indicated that ADP elicits phospholipase C activation while the same effect could not be detected in human platelets by the same authors [24].

In this study, we investigated the *ex vivo* effect of PCR 4099 on phospholipase C and calcium mobilization induced by ADP in washed rat platelet.

## MATERIALS AND METHODS

*Materials.* Adenosine diphosphate (ADP) was purchased from Boehringer (Mannheim, F.R.G.). Bovine thrombin (63 NIH units/mg protein) was provided by Hoffman-La Roche (Basel, Switzerland). Quin 2 AM (quin 2 acetoxymethylester), creatine phosphate (CP) and creatine phosphokinase (CPK, 100–150 units/mg protein) were obtained from Sigma (St Louis, MO). Aspirin (ASA), lysine salt, was provided by Egic-Joullie Laboratories (Montargis, France). PCR 4099 (*d,l* methyl(2-chlorophenyl) - 5 - (4,5,6,7 - tetrahydrothieno(3,2 - *c*)pyridyl)acetate, hydrochloride, hydrate) was synthesized by Sanofi Recherche (Toulouse, France). [$^{32}$P]-Orthophosphate was from the Radiochemical Centre (Amersham, U.K.).

*Animals and drug administration.* Female CD-COBS rats (Charles Rivers) weighing 220–250 g were used. PCR 4099 dispersed in 5% arabic gum solution was orally administered by gavage, at the dose of 100 mg/kg/day for 3 days. The last administration preceded by 1 hr blood processing.

*Preparation of platelet suspensions.* Blood was collected from abdominal aorta under ether anaesthesia into 0.1 vol. citric acid, sodium citrate, dextrose (7 mM, 93 mM, 140 mM, respectively), pH 6.8. Platelet-rich plasma was obtained by a rapid procedure, i.e. centrifugation at 1000 *g* for 2 min and platelets were resuspended in a modified Tangen buffer pH. 7.4 containing 145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 5.5 mM glucose, 15 mM Hepes and 1 mg/ml bovine serum albumin [25]. The platelet count was adjusted to $8 \times 10^8$ cells/ml.

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§ Abbreviations used: ADP, adenosine diphosphate; $IP_3$, inositol-1,4,5-trisphosphate; PA, phosphatidic acid; $PIP_2$, phospatidylinositol-4,5-bisphosphate; PIP, phosphatidylinositol 4-phosphate; PI, phosphatidyl-inositol; ASA, acetyl salicylic acid; CP, creatine phosphate; CPK, creatine phosphokinase.

*Measurement of cytoplasmic free calcium concentration.* This was performed by the fluorimetric method of Rink *et al.* [26], using a Jobin-Yvon spectrofluorimeter (model JY 3C). Platelet suspension in modified Tangen buffer [25] was incubated for 30 min at 37° with 15 $\mu$M quin 2-AM. ASA (0.5 mM) was added 15 min before the end of the incubation. Platelets were then centrifuged for 15 min at 1000 *g* and washed twice to remove unincorporated quin 2. Platelets were diluted to approximately $10^8$ cells/ml and placed in a quartz cuvette at 37°. Fluorescence (excitation at 339 nm, emission at 500 nm) was measured before and after the addition of $CaCl_2$ (1 mM) or EGTA (1 mM) and ADP at various concentrations. The maximum fluorescence ($F_{max}$) was obtained using digitonin (50 $\mu$M) which disrupts plasma membrane, in the presence of 1 mM $CaCl_2$. The minimum fluorescence ($F_{min}$) was then obtained after adding 3 mM EGTA and 10 mM Tris [27]. Cytoplasmic concentration of resting and ADP exposed platelets were calculated using the equation from Tsien *et al.* [28].

*Preparation of* [$^{32}$P]o-*phosphate-labelled platelets.* Platelets were prelabelled in the first washing solution ($8 \times 10^8$ cells/ml) at 37° for 45 min, with [$^{32}$P]*o*-phosphate (25 $\mu$Ci/ml). ASA (0.5 mM) was added 15 min before the end of the incubation. The unincorporated label was removed by centrifuging and washing platelets twice in a modified Tangen buffer [25]. Platelets were finally resuspended in the same buffer to a final density of $8 \times 10^8$ cells/ml.

*Stimulation of prelabelled platelets and analytical procedures.* Platelet suspensions (0.5 ml aliquots) were equilibrated for 1 min at 37° after addition of EGTA (1 mM). ADP at various concentrations or thrombin (0.2 IU/ml, final concentration) was added and the incubation was continued for 1 min. When thrombin was used, creatine phosphate (CP, 5 mM) and creatine phosphokinase (CPK, 40 IU/ml) were added prior to stimulation. The reaction was blocked by addition of 1 ml of chloroform/methanol (1/1, v/v) and 0.05 ml of 200 mM EDTA pH 7.4.

Lipids were extracted according to Bligh and Dyer [29] after acidification with 10 M HCl [30]. Phospholipids were separated by monodimensional thin-layer chromatography on oxalate-impregnated silicagel plates (Merck, Darmstadt), F.R.G.) using the procedure of Jolles *et al.* [31]. After autoradiography, the radioactive spot corresponding to phosphatidic acid was scaped off and submitted to liquid scintillation counting.

*Aggregation and shape change measurements.* Before all experiments, platelets were checked for aggregation and shape change by a standard turbidimetric technique [32] at 37° using a Coultronics aggregometer (Chronolog Corp. Harveston, PA).

## RESULTS

In all experiments, platelets were treated with aspirin in order to eliminate any participation of cyclooxygenase products.

### *Effects of PCR 4099 on platelet aggregation and shape change induced by ADP*

After administration of PCR 4099, aggregation of washed rat platelets in response to 10 $\mu$M ADP was suppressed while shape change was unaffected (Fig. 1A). In the absence of extracellular calcium, only shape change could be detected in both control and PCR 4099-treated platelets (Fig. 1B).

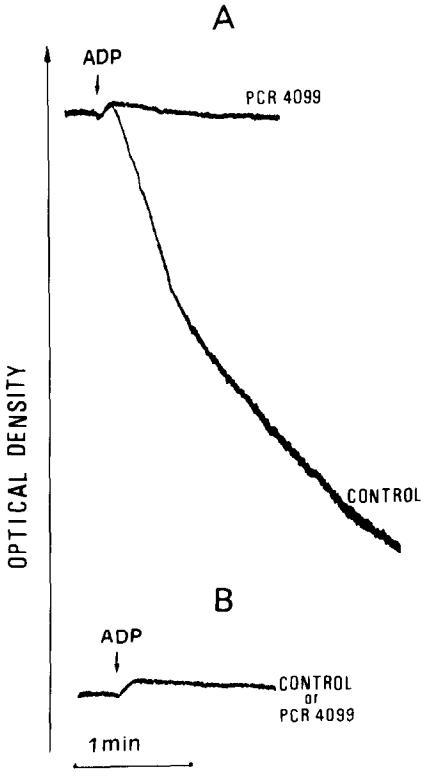


Fig. 1. Effects of PCR 4099 administration on washed rat platelet aggregation and shape change induced by ADP: (A) in the presence of 1 mM $Ca^{2+}$; (B) in the presence of 1 mM EGTA. Data are from one experiment representative of six different experiments with identical results.

### *Effect of PCR 4099 on calcium movements*

Figure 2 represents typical records of calcium movements, monitored by quin 2 fluorescence changes in platelets stimulated by 10 $\mu$M ADP. In the presence of 1 mM extracellular calcium, where the large fluorescence signal is mainly due to $Ca^{2+}$ influx, cytoplasmic free calcium concentration rose rapidly from the basal level of about 100 nm to 550 nM in both control and PCR 4099-treated platelets. In the absence of external calcium, where the fluorescence signal corresponds to $Ca^{2+}$ mobilization from dense tubular system, ADP stimulation induced an increase of $[Ca^{2+}]_i$ in the control platelets from the basal level of 50 nM to 140 nM (Fig. 2A), while $Ca^{2+}$ mobilization was almost abolished in PCR 4099 treated platelets (Fig. 2B).

The ADP dose–response curves in the presence of external $Ca^{2+}$ (Fig. 3A) showed that PCR 4099 was virtually without any effect against ADP-induced $Ca^{2+}$ influx in washed rat platelets, whatever the ADP concentrations used, and maximal cytoplasmic free calcium concentration was obtained with 2.5 $\mu$M

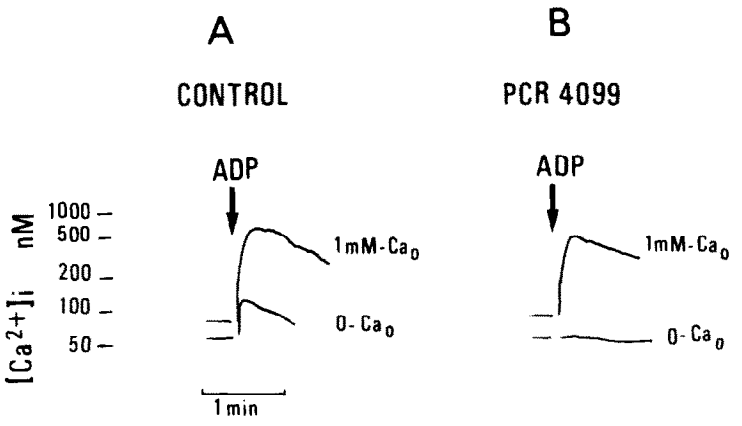


Fig. 2. Effects of PCR 4099 on calcium movements induced by 10 μM ADP. Data are from one experiment representative of six different experiments with identical results.

ADP, in both control and drug-treated platelets. In the absence of external $Ca^{2+}$, PCR 4099 treatment suppressed $Ca^{2+}$ mobilization induced by all doses of ADP used (Fig. 3B).

*Effects of PCR 4099 on phosphatidic acid production*

As illustrated in Fig. 4, ADP caused an increase in $^{32}P$-labelled PA. A paired Student's *t*-test of these data showed that at all ADP doses the increase was statistically significant at least at 98%. PCR 4099 significantly inhibited the ADP-induced PA production by about 45% ($P < 0.05$, $N = 3$).

It has been shown that platelet aggregation and secretion induced by low thrombin concentrations is highly dependent on released ADP [3, 33]. Under these conditions (0.2 IU/ml thrombin) PCR 4099 significantly inhibited PA production by about 55% ($P < 0.02$, $N = 3$) (see Table 1). The same effect was obtained upon *in vitro* addition of CP/CPK, in both control and PCR 4099 treated platelets (Table 1).

## DISCUSSION

In a previous study, we demonstrated that ticlopidine and its analogue PCR 4099, exert their antiplatelet effect against released ADP [3]. It then seemed interesting to investigate the effects of these drugs on the biochemical steps involved in platelet stimulation by ADP. Platelets obtained after PCR 4099 administration exhibited impaired aggregation but had normal shape change in response to ADP. These observations are consistent with previously

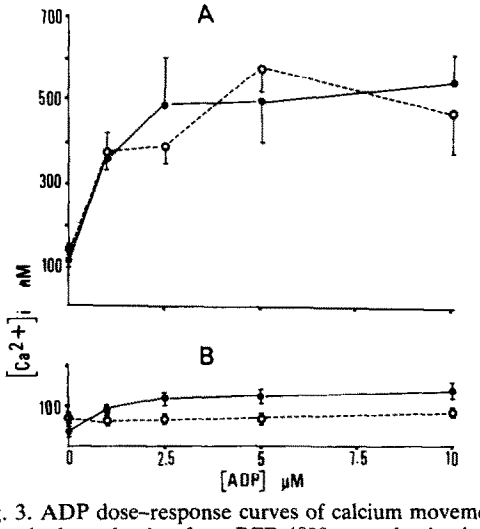


Fig. 3. ADP dose–response curves of calcium movements in washed rat platelets from PCR 4099 treated animals and from control: (A) in the presence of 1 mM $Ca^{2+}$; (B) in the presence of 1 mM EGTA; ●——●, control; ○----○, treated. Results are mean ± SEM of six experiments.

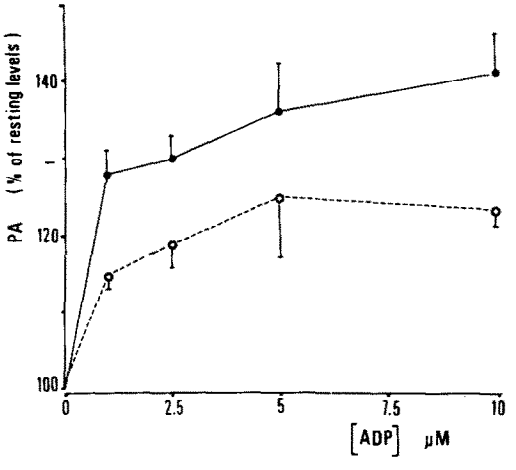


Fig. 4. Effect of PCR 4099 on phosphatitic acid production induced by ADP in washed rat platelets: ●——●, control; ○----○, treated. Results are mean ± SEM of three experiments.

Table 1. Effect of PCR 4099 on phosphatidic acid (PA) production induced by thrombin 0.2 IU/ml compared to the effect of CP/CPK *in vitro* (mean ± SEM; N = 3)

| Treatment | PA (% of resting levels) |
|---|---|
| Control | 453 ± 26 |
| PCR 4099 | 249 ± 39** |
| CP/CPK | 264 ± 55* |
| PCR 4099 + CP/CPK | 247 ± 62* |

Unpaired Student's *t*-test: *P < 0.05; **P < 0.02 (versus control).

published data [34] and suggest that PCR 4099 does not affect the interaction of ADP with its receptor at least for that concerning shape change. Determination of $Ca^{2+}$ movements triggered by ADP revealed that PCR 4099 leaves intact $Ca^{2+}$ influx across plasma membrane but strongly inhibits $Ca^{2+}$ mobilization from internal stores. A suppression by ticlopidine of thrombin-induced $Ca^{2+}$ influx has been recently reported by Morikawa *et al.* [35], but at this thrombin concentration (1 IU/ml), we were unable to observe any change (not shown). Our results are not so surprising since PCR 4099 like ticlopidine, does not inhibit platelet activation induced by high thrombin concentrations [3]. The reasons for such a discrepancy remain presently unclear.

Since $Ca^{2+}$ mobilization is due to $IP_3$ generation [19, 20], we then investigated the effects of PCR 4099 on phospholipase C activation induced by ADP. This was performed by measuring [$^{32}$P]PA production, the latter compound being formed by phosphorylation of diacylglycerol. Although this method does not enable us to identify the substrate used by phospholipase C ($PIP_2$, PIP, PI or other phospholipids), it remains much more sensitive than determination of $IP_3$, which requires previous labelling of platelet inositol-phospholipids with large amount of [$^3$H]inositol. This is particularly critical in the case of ADP, for which controversial results have been reported [17, 18, 21, 23]. Under these conditions, we observed that inhibition of $Ca^{2+}$ mobilization induced by PCR 4099 was accompanied by a significant loss of [$^{32}$P]PA labelling. So our results support the view that $Ca^{2+}$ mobilization induced by ADP requires phospholipase C activation and generation of $IP_3$. Such an observation is in agreement with the data of Daniel *et al.* [18], but contrasts with other reports [17, 21–23]. However, Vickers *et al.* [23] proposed that ADP is only able to stimulate phospholipase C action against PIP and PI in rabbit platelets. Our results would also partially support their conclusion, since some PA formation was still observed under conditions where $Ca^{2+}$ mobilization was completely abolished.

Table 2. Effects of PCR 4099 on various biochemical and functional events evoked by ADP in rat platelets

| Effects of ADP | Inhibition by PCR 4099 (%) |
|---|---|
| $Ca^{2+}$ influx | 0 |
| $Ca^{2+}$ mobilization | 100 |
| PA synthesis | 45 |
| Shape change | 0 |
| Fibrinogen binding | 50 to 90* |
| Aggregation | 100 |

* Gachet *et al.* [39].

Further evidence for a possible role of ADP in activating platelet phospholipase C is brought about by the observation that the enzymatic ADP-removing system CP/CPK depressed [$^{32}$P]PA production induced by thrombin by almost 50%. As seen previously for platelet aggregation and secretion [3], such an inhibition is indistinguishable from the effect of PCR 4099. Furthermore, no synergism appeared between *in vitro* addition of CP/CPK and *ex vivo* treatment with PCR 4099. This strongly suggests that PCR 4099 antagonizes the effect of released ADP. Actually, such an effect of ADP on $Ca^{2+}$ mobilization probably involving $IP_3$ generation might not be due to ADP itself, but rather to the binding of fibrinogen to glycoprotein IIb/IIIa complex (in fact to the conformational change of glycoprotein IIb/IIIa, since fibrinogen does not bind in the presence of EGTA, under which conditions $Ca^{2+}$ mobilization still occurs). In this respect, it is worth remembering that, at low thrombin concentrations, fibrinogen binding to platelets is almost entirely due to released ADP [33]. Such a proposal was recently presented by Banga *et al.* [36], but these authors suggested that phospholipase C activation following fibrinogen interaction with its receptor actually involves phospholipase $A_2$ stimulation and thromboxane $A_2$ generation. This is certainly not the case here, since all the experiments were performed with ASA-treated platelet. Such a discrepancy might account for an animal species difference, since rat platelets remain insensitive to thromboxane $A_2$ [3, 37, 38].

In summary, the main data obtained in the present study are reported in Table 2. Some clear conclusions can be drawn from our observations. (1) $Ca^{2+}$ influx and $Ca^{2+}$ mobilization induced by ADP are quite different processes, showing different sensitivity to PCR 4099 and suggesting the involvement of two different receptors (or two different transduction mechanisms). (2) Shape change can occur normally without any increase of cytoplasmic free $Ca^{2+}$, i.e. in the presence of EGTA and with platelets treated *ex vivo* with PCR 4099. Such an observation is in agreement with a recent study of Hallam *et al.* [40], but the mechanism of shape change still remains to be understood. One can notice anyway that, under these conditions, some residual PA synthesis still occurs. Although we have not yet any evidence for the nature of phospholipids (probably not $PIP_2$) used as a substrate by phospholipase C, such an observation might offer some clue to further explore the biochemical mechanism of platelet shape change. (3) A close relationship seems to exist between exposure of fibrinogen receptors, $Ca^{2+}$ mobilization and part of PA synthesis. As discussed above, the latter two events might correspond to $PIP_2$ hydrolysis.

## REFERENCES


1. Panak E, Maffrand JP, Picard-Fraire C, Vallée E, Blanchard J and Roncucci R, Ticlopidine: a promise for the prevention and treatment of thrombosis and complications. *Haemostasis* **13** (suppl. 1): 1–54, 1983.
2. Maffrand JP, Vallée E, Bernat A, Delebassée D, Millou E, Tissinier A and Roncucci R, Animal pharmacology of PCR 4099, a new thienopyridine compound. *Thromb Haemost.* **54**: 133, 1985.
3. Feliste R, Delebassée D, Simon MF, Chap H, Defreyn G, Vallée E, Douste-Blazy L and Maffrand JP, Broad spectrum anti-platelet activity of ticlopidine and PCR 4099 involves the suppression of the effects of released ADP. *Thromb Res* **48**: 403–415, 1987.
4. Gaarder A, Jonsen J, Laland S, Hellem A and Owren PA, Adenosine Diphosphate in red cells as a factor in the adhesiveness of human blood platelets. *Nature (Lond)* **192**: 531–532, 1961.
5. Born GVR, Bergquist D and Arfors KE, Evidence for inhibition of platelet activation in blood by a drug effect on erythrocytes. *Nature (Lond)* **259**: 233–235 1976.
6. Born GVR and Wehmeier A, Inhibition of platelet thrombus formation by chlorpromazine acting to diminish haemolysis. *Nature (Lond)* **282**: 212–213, 1979.
7. Bergquist D and Arfors KE, Haemostatic platelet plug formation in the isolated rabbit mesenteric preparation. An analysis of red blood cell participation. *Thromb. Haemost.* **44**: 6–8, 1980.
8. Saniabadi AR, Lowe GDD, Barbenel JC and Forbes CD, A comparison of spontaneous platelet aggregation in whole blood with platelet rich plasma: additional evidence for the role of ADP. *Thromb Haemost* **51**: 115–118, 1984.
9. Born GVR, Observations on the change in shape of blood platelets brought about by adenosine diphosphate. *J Physiol (Lond)* **209**: 487–511, 1970.
10. Bennett JS and Vilaire G, Exposure of platelet fibrinogen receptors by ADP and epinephrine. *J Clin Invest* **64**: 1393–1401, 1979.
11. Marguerie GA, Edgington TS and Plow EF, Interaction of Fibrinogen with its platelet receptor as part of a multistep reaction in ADP-induced platelet aggregation. *J Biol Chem* **255**: 154–161, 1980.
12. Hallam TJ and Rink TJ, Responses to adenosine diphosphate in human platelets loaded with the fluorescent calcium indicator Quin 2. *J Physiol (Lond)* **368**: 131–146, 1985.
13. Born GVR, Uptake of adenosine and of adenosine diphosphate by human blood platelets. *Nature (Lond)* **206**: 1121–1122, 1965.
14. Boullin DJ, Gree AR and Price KS, The mechanism of adenosine diphosphate induced platelet aggregation: binding to platelet receptors and inhibition of binding and aggregation by prostaglandin E. *J Physiol* **221**: 415–426, 1972.
15. Born GVR and Feinberg H, Binding of adenosine diphosphate to intact human platelets. *J Physiol (Lond)* **251**: 803–816, 1975.
16. Lips JP, Sixma JJ and Schiphorst ME, Binding of adenosine diphosphate to human blood platelets and to human blood platelet membranes. *Biochim Biophys Acta* **628**: 451–467, 1980.
17. Fisher GJ, Bakshian S and Baldassare JJ, Activation of human platelets by ADP causes a rapid rise in cytosolic free calcium without hydrolysis of phosphatidylinositol-4,5-bisphosphate. *Biochem Biophys Res Commun* **129**: 958–964, 1985.
18. Daniel JL, Dangelmaier CA, Selak M and Smith JB, ADP stimulates $IP_3$ formation in human platelets. *FEBS Lett* **206**: 299–303, 1986.
19. Berridge MJ, Inositol trisphosphate and diacylglycerol as second messengers. *Biochem J* **220**: 345–360, 1984.
20. Brass LF and Joseph SK, A role for inositol triphosphate in intracellular $Ca^{2+}$ mobilization and granule secretion in platelets. *J Biol Chem* **260**: 15172–15179, 1985.
21. Vickers JD, Kinlough-Rathbone RL and Mustard JF, Changes in phosphatidylinositol-4,5-bisphosphate and other phospholipids 10 seconds after stimulation of washed rabbit platelets with ADP. *Blood* **60**: 1247–1250, 1982.
22. Leung NL, Vickers JD, Kinlough-Rathbone RL, Reimers HJ and Mustard JF, ADP-induced changes in [$^{32}$P]phosphate labeling of phosphatidylinositol-4,5-bisphosphate in washed rabbit platelets made refractory by prior ADP stimulation. *Biochem Biophys Res Commun* **113**: 483–490, 1983.
23. Vickers JD, Kinlough-Rathbone RL and Mustard JF, The decrease in phosphatidylinositol 4,5-bisphosphate in ADP-stimulated washed rabbit platelets is not primarily due to phospholipase C activation. *Biochem J* **237**: 327–332, 1986.
24. Drummond AH and MacIntyre DE, Platelet inositol lipid metabolism and calcium flux. In: *Platelets in Biology and Pathology III* (Eds. MacIntyre DE and Gordon JI), pp. 373–431. Elsevier (Biochemical Division), Amsterdam, 1987.
25. Tangen O, Andrae ML and Nilsson BE, Nucleotide leakage from platelets in artificial media: prevention by albumin and other macromolecules and relation to ADP-induced platelet aggregation. *Scand J Haematol* **11**: 241–248, 1973.
26. Rink TJ, Smith SV and Tsien RY, Cytoplasmic free $Ca^{2+}$ in human platelets: $Ca^{2+}$ thresholds and Ca-independent activation for shape change and secretion. *FEBS Lett* **148**: 21–26, 1982.
27. Rittenhouse SE and Horne WC, Ionomycin can elevate intraplatelet $Ca^{2+}$ and activate phospholipase A without activating phospholipase C. *Biochim Biophys Res Commun* **123**: 393–397, 1984.
28. Tsien RY, Pozzan T and Rink TJ, Calcium homeostasis in intact lymphocytes: cytoplasmic free calcium monitored with a new intracellularly trapped fluorescent indicator. *J Cell Biol* **94**: 325–334, 1982.
29. Bligh EG and Dyer WJ, A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**: 911–918, 1959.
30. Mauco G, Chap H, Simon MF and Douste-Blazy L, Phosphatidic and lysophosphatidic acid production in phospholipase C- and thrombin-treated platelets. Possible involvement of a platelet lipase. *Biochimie* **60**: 653–661, 1978.
31. Jolles J, Schrama LH and Gispen WH, Calcium-dependent turnover of brain polyphosphoinositides *in vitro* after prelabelling *in vivo*. *Biochim Biophys Acta* **666**: 90–98, 1981.
32. Born GVR, Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature (Lond)* **194**: 927–929, 1962.
33. Plow EF and Marguerie GA, Participation of ADP in the binding of fibrinogen to thrombin-stimulated platelets. *Blood* **56**: 553–555, 1980.
34. Di Minno G, Gerbone AM, Mattioli PL, Turco S, Iovine C and Mancini M, Functionally thrombasthenic state in normal platelets following the administration of ticlopidine. *J Clin Invest* **75**: 328–338, 1985.
35. Morikawa M, Kojima T, Inoue M and Tsuboi M, Comparison of the inhibitory effects of aspirin and ticlopidine on platelet aggregation and $Ca^{2+}$ mobilization in rat platelets. *Res Commun Chem Pathol Pharmacol* **54**: 275–278, 1986.
36. Banga HS, Simons ER, Brass LF and Rittenhouse SE, Activation of phospholipase A and C in human platelets

exposed to epinephrine: Role of glycoproteins IIb/IIIa and dual role of epinephrine. *Proc Natl Acad Sci USA* **83**: 9197–9201, 1986.
37. Bult H and Bonta IL, Rat platelets aggregate in the absence of endogenous precursors of prostaglandin endoperoxides, *Nature (Lond)* **264**: 449–451, 1976.
38. Hwang DH, Aggregation and inhibition of rat platelets, and the formation of endoperoxide metabolites. *Prostagl Med* **5**: 163–173, 1980.
39. Gachet C, Stierlé A, Bouloux C, Maffrand JP and Cazenave JP, The thienopyridine PCR 4099 inhibits the ADP aggregation pathway of human platelets by interfering with the binding of fibrinogen to the glycoprotein IIb–IIIa complex. *Thromb Haemost* **58**: 221, abstract, 782, 1987.
40. Hallam TJ, Daniel JL, Kendrick-Jones J and Rink TJ, Relationship between cytoplasmic free calcium and myosin light chain phosphorylation in intact platelets. *Biochem J* **232**: 373–377, 1985.