Agents that elevate platelet cAMP stimulate the formation of phosphatidylinositol 4-phosphate in intact human platelets

D. de Chaffoy de Courcelles, P. Roevens and H. van Belle

Department of Biochemistry, Janssen Pharmaceutica, B-2340 Beerse, Belgium

Received 17 October 1985

The present study investigates the effect of compounds that are known to elevate cAMP on the phospholipid metabolism of platelets. Prostaglandin E_1, forskolin and isobutylmethylxanthine induce an increase in [^{32}P]-phosphatidylinositol 4-phosphate (PIP) in platelets prelabelled with [^{32}P]orthophosphate. Possible roles of this phenomenon are discussed in view of the inhibitory effect of cAMP elevation on platelet activation.

Polyphosphoinositide *Prostaglandin E_1* *Forskolin* *Isobutylmethylxanthine* *Platelet*

1. INTRODUCTION

Inositol phospholipids are involved in the signal transducing system of many platelet stimuli (review [1,2]). Phosphatidylinositol 4,5-bisphosphate (PIP_2) is apparently of major importance. Its phosphodiesteratic cleavage yields diacylglycerol and inositol trisphosphate. Diacylglycerol activates the C kinase which plays an important role in the physiological response [2]. Inositol trisphosphate has been demonstrated to be a potential mediator of Ca^{2+} release from intracellular Ca stores [1].

Phosphatidylinositol 4-phosphate (PIP) is an intermediate between phosphatidylinositol (PI) and PIP_2. In [3,4] we showed that direct activation of C kinase by 1-oleoyl-2-acetylglycerol (OAG) and 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) stimulates ^{32}P incorporation predominantly in PIP. Authors in [5] demonstrated that the increase in incorporation corresponded to an increase in mass.

More recently the direct activation of C kinase was reported to have a negative feedback control over agonist-induced hydrolysis of inositol phospholipids. Treatment of cellular systems with OAG or TPA inhibited the effects of agonists on the primary receptor-coupled biochemical events as there are the activation of phospholipase C [7–11] and the increase in intracellular free Ca^{2+} [7–9,11,12].

Inhibition of the same events occurs when platelets are treated with agents known to elevate their cAMP content [13–20].

In analogy with our findings on OAG and TPA-induced PIP formation, we investigated whether agents known to elevate platelet cAMP also increase [^{32}P]PIP formation.

2. MATERIALS AND METHODS

Prostaglandin E_1 (PGE_1) and phospholipid reference product were obtained from Sigma (St. Louis, USA). IBMX (isobutylmethylxanthine) was from Janssen Chimica (Beerse) and forskolin (FK) from Calbiochem (La Jolla, USA). Silica gel 60-precoated plastic sheets were from E. Merck, FRG. [^{32}P]Orthophosphate was obtained from Amersham (England). Proteins for M_r determinations on SDS-polyacrylamide gels were from Bio-Rad. Platelet preparation, ^{32}P labelling of platelets, and lipid and protein analyses were performed exactly as in [21].

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

The control ^{32}P incorporation was always determined in the presence of the solvent for the compounds tested (1‰ ethanol for PGE_1 and FK, 1% DMSO for IBMX). Preliminary experiments had shown that the solvents alone had no influence on ^{32}P incorporation. Within the different experiments the control labelling in the PIP pool varied between 35000 and 100000 cpm. Within the time course of an experiment the incorporation did not significantly change [21]. The changes with addition of a compound are expressed as % of the control ^{32}P incorporation.

3. RESULTS

On addition of PGE_1 (10^{-6} M), FK (10^{-5} M) and IBMX (10^{-3} M) the ^{32}P incorporation in PIP increased (fig.1). None of the compounds significantly altered ^{32}P incorporation in the other labelled lipid fractions (PIP_2, PI, phosphatidic acid, phosphatidylcholine and lysophosphatidylinositol) (not shown). The onset of phosphorylation differed for the 3 compounds. Phosphorylation induced by PGE_1 is more rapid than that of FK (fig.1); after 30 s the former reached about 70% of its maximum whereas the latter attained only 30–35%. [^{32}P]PIP formation induced by IBMX is low and more delayed (fig.1), being half maximal only after about 1 min.

Fig.2 illustrates that the changes in PIP induced by PGE_1 and FK are clearly concentration-dependent, PGE_1 being the most potent compound. The changes in [^{32}P]PIP provoked by IBMX were too small to assess accurately a dose dependency.

PGE_1, FK and IBMX induce phosphorylation of a 50 kDa, a 27 kDa and a 24 kDa protein (not shown) most probably by the activation of a cAMP-dependent protein kinase. Similar changes in protein phosphorylation patterns were found by others [22–24]. The 40 kDa protein phosphorylation (substrate for C kinase) was not affected [25].

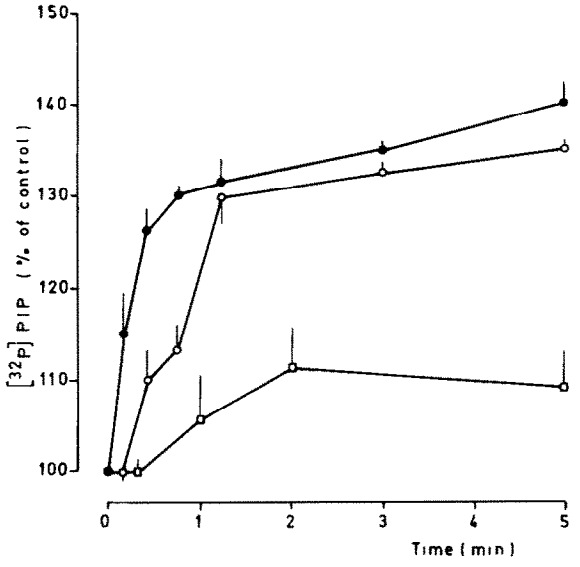

Fig.1. Changes in [^{32}P]PIP after addition of PGE_1, FK or IBMX. PGE_1 (10^{-6} M) (●—●), FK (10^{-5} M) (○—○) and IBMX (10^{-3} M) (□—□) were added to platelets prelabelled with [^{32}P]orthophosphate. At the indicated times, platelet samples were taken and incubation was stopped by adding the platelets to organic solvent (see section 2). Points represent the means ± SE of duplicate samples from 4 (for PGE_1 and FK) and 2 (for IBMX) experiments. The control ^{32}P incorporation was taken as 100% for each experiment (see section 2).

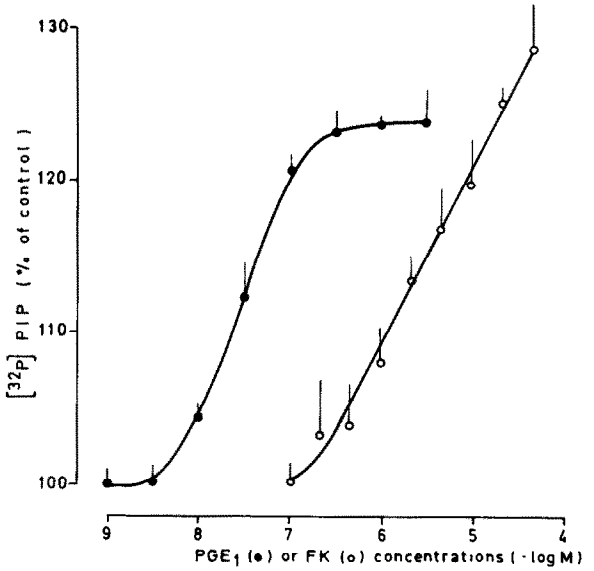

Fig.2. Concentration-dependent changes in [^{32}P]PIP. PGE_1 and FK were added to platelets prelabelled with [^{32}P]orthophosphate. After 25 s (for PGE_1) and 1 min (for FK) the incubation was stopped as described in the legend to fig.1. Points represent the means ± SE of duplicate samples from 4 (for PGE_1) and 3 (for FK) experiments. The control ^{32}P incorporation was taken as 100% for each experiment.

4. DISCUSSION

Platelets have a bidirectional control system for transduction of an extracellular signal to the cell interior. Platelet activation by excitatory stimuli, that use Ca^{2+} and diacylglycerol as messengers, is antagonized by inhibitory platelet stimuli known to provoke a rise in cAMP [13–20] such as prostaglandins (i.e. PGE_1, PGD_2, PGI_2), FK and IBMX. Although acting through a different mechanism to elevate cAMP, the latter compounds all induce an increase in the [^{32}P]PIP levels in the platelet.

The complex metabolism of the polyphosphoinositides does not allow one to assign unequivocally this alteration to a specific enzymatic step. Increased [^{32}P]PIP formation can be explained through phosphorylation of PI as we discussed in [4]. This statement is in agreement with findings that the cAMP-dependent protein kinase stimulates the formation of polyphosphoinositides in plasma membranes from platelets [26] and the formation of PIP in plasma membranes from pig granulocytes [27]. Since an elevation of platelet PIP levels in a resting platelet does not appear physiologically relevant it is tempting to look for a separate role for PIP besides being an intermediate metabolite between PI and PIP_2. In this context the finding of authors in [28,29] that PIP might be involved in Ca transport ATPases is attractive. Both activation of the C kinase [9] and addition of PGE_1 [20] have been shown to stimulate Ca^{2+} extrusion from the platelet cytoplasm. This lowering of a steady-state level of Ca^{2+} (second or third messenger) might evidently contribute to the inhibitory effects seen in these conditions.

Alternatively, a cAMP-induced shift of the PIP $\rightleftharpoons$ PIP_2 equilibrium to the left can explain an accumulation of [^{32}P]PIP. Since the inositol lipid cycle [$PIP_2 \longrightarrow$ DAG ($+ Ip_3$) $\longrightarrow$ PA $\longrightarrow$PI $\longrightarrow$ PIP $\longrightarrow$ PIP_2] does occur in the resting platelet [19,30], this would lead to concomitant lower steady-state levels of metabolites distal to PIP in inositol lipid cycle. Of major importance in the inhibitory effect of agents that elevate platelet cAMP would be the lowering in PIP_2 as substrate for agonist-induced phospholipase C and the decrease in steady-state levels of the putative second messengers (Ip_3 and DAG). Small changes in the equilibrium PIP $\rightleftharpoons$ PIP_2 would have an amplificatory inhibitory effect on platelet activation; also, the excitatory agonist-induced increase as the basal levels of the second messenger would be lowered.

In conclusion, we found that agents known to elevate platelet cAMP induce an increase in [^{32}P]PIP in the human platelet. Complete analogy at the level of signal transduction is now found between the negative feedback control of platelet activation by the C kinase and the inhibitory effect of agents that elevate platelet cAMP since both phenomena coincide with (i) a decrease in cytosolic Ca^{2+}, (ii) a decrease in phospholipase C activity and (iii) an increase in PIP formation. A possible role for the latter remains to be elucidated.

REFERENCES

[1] Berridge, M.J. and Irvine, R.F. (1984) Nature 312, 315–321.
[2] Nishizuka, Y. (1984) Science 225, 1365–1370.
[3] De Chaffoy de Courcelles, D., Roevens, P. and Van Belle, H. (1984) FEBS Lett. 173, 389–393.
[4] De Chaffoy de Courcelles, D., Roevens, P. and Van Belle, H. (1984) Biochem. Biophys. Res. Commun. 123, 589–595.
[5] Halenda, S.P. and Feinstein, M.B. (1984) Biochem. Biophys. Res. Commun. 124, 507–513.
[6] Labarca, R., Janowsky, A., Patel, J. and Paul, S.M. (1984) Biochem. Biophys. Res. Commun. 123, 703–709.
[7] MacIntyre, D.E., McNical, A. and Drummond, A.M. (1985) FEBS Lett. 180, 160–164.
[8] Watson, S.P. and Lapetina, E.G. (1985) Proc. Natl. Acad. Sci. USA 82, 2623–2626.
[9] Zavoico, G.B., Halenda, S.P., Sha'afi, R.I. and Feinstein, M.B. (1985) Proc. Natl. Acad. Sci. USA 82, 3859–3862.
[10] Rittenhouse, S.E. and Sason, J.P. (1985) J. Biol. Chem. 260, 8657–8660.
[11] Lynch, C.J., Charest, R., Bocckino, S.B., Exton, J.M. and Blackmore, P.F. (1985) J. Biol. Chem. 260, 2844–2851.
[12] Cooper, R.H., Coll, K.E. and Williamson, J.R. (1985) J. Biol. Chem. 260, 3281–3288.
[13] Rittenhouse-Simmons, S. (1979) J. Clin. Invest. 63, 580–587.
[14] Billah, M.M., Lapetina, E.G. and Cuatrecasas, P. (1979) Biochem. Biophys. Res. Commun. 90, 92–98.
[15] Billah, M.M. and Lapetina, E.G. (1983) Proc. Natl. Acad. Sci. USA 80, 965–968.

[16] Hallam, T.J., Rink, T.J. and Sanchez, A.J. (1983) J. Physiol. 343, 98P.
[17] Feinstein, M.B., Egan, J.J., Sha'afi, R.I. and White, J. (1983) Biochem. Biophys. Res. Commun. 113, 558–604.
[18] Zavoico, G.B. and Feinstein, M.B. (1984) Biochem. Biophys. Res. Commun. 120, 579–585.
[19] Watson, S.P., McConnell, R.J. and Lapetina, E.G. (1984) J. Biol. Chem. 259, 13199–13203.
[20] Affolter, H., Erne, P., Bürgisser, E. and Pletscher, A. (1984) Naunyn-Schmiedeberg's Arch. Pharmacol. 325, 337–342.
[21] De Chaffoy de Courcelles, D., Roevens, P. and Van Belle, H. (1985) J. Biol. Chem., in press.
[22] Haslam, R.J., Lynham, J.A. and Fox, J.E.B. (1979) Biochem. J. 178, 397–406.
[23] Nishizuka, Y. (1983) Phil. Trans. R. Soc. Lond. Ser. B 302, 101–112.
[24] Lapetina, E.G., Watson, S.P. and Cuatrecasas, P. (1984) Proc. Natl. Acad. Sci. USA 81, 7431–7435.
[25] De Chaffoy de Courcelles, D., Roevens, P., Fraipont, E., Gabriëls, L. and Van Belle, H. (1985) submitted.
[26] Enyedi, A., Faragó, A., Sarkadi, B., Szász, I. and Gárdos, G. (1983) FEBS Lett. 161, 158–162.
[27] Farkas, G., Enyedi, A., Sarkadi, B., Gárdos, G., Nagy, Z. and Faragó, A. (1984) Biochem. Biophys. Res. Commun. 124, 871–876.
[28] Varsanyi, M., Tölle, K.-G., Heilmayer, L.M.G., Dawson, R.M.C. and Irvine, R.F. (1983) EMBO J. 2, 1543–1548.
[29] Choquette, D., Hakim, G., Filoteo, A.G., Plishker, G.A., Bostwick, J.R. and Penniston, J.T. (1984) Biochem. Biophys. Res. Commun. 125, 908–915.
[30] Agranoff, B.W., Murhy, P. and Seguin, E.B. (1983) J. Biol. Chem. 258, 2076–2078.