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# PDGF MODIFIES PHOSPHOINOSITIDE METABOLISM AND INHIBITS AGGREGATION AND RELEASE IN HUMAN PLATELETS

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SUMMARY : While platelet derived growth factor (PDGF) did not induce any platelet aggregation nor secretion, it modified the polyphosphoinositide metabolism of human platelets prelabeled with $^{32}$P-orthophosphate. We found a decrease of $^{32}$P associated with phosphatidylinositol 4,5 bisphosphate after 3 min, with parallel increase of $^{32}$P-phosphatidylinositol 4 phosphate and $^{32}$P-phosphatidylinositol using 100 ng/ml of PDGF. This modification was PDGF concentration dependent. PDGF inhibited thrombin and collagen induced platelet aggregation and $^{14}$C-serotonin release in a dose dependent manner, but was without effect when arachidonic acid was used. These results suggest that PDGF (i) stimulated the hydrolysis of polyphosphoinositides (ii) and could exert a negative feedback control on platelet activation induced by thrombin or collagen. 

PDGF contained in platelet α granules is a potent mitogen for fibroblasts and smooth muscle cells. The mitogenic effect has been mainly investigated using fibroblasts 3T3. Cell responses to PDGF binding are (i) increase of phosphorylation of the PDGF-receptor and tyrosine kinase activity (1, 2) (ii) modification of transport ions (3) (iii) changes in phosphatidylinositol and arachidonic acid metabolism (4, 5). Recent work has shown that platelet activation is associated with rapid PPI breakdown and described a parallel time course of $PIP_2$ breakdown with dense body release in thrombin activated platelets (6). It has been demonstrated that potent tumor promoter phorbol esters increased the incorporation of $^{32}$P-phosphoinositides into PIP and $PIP_2$

Abbreviations used : Platelet derived growth factor, (PDGF) ; polyphosphoinositides, (PPI) ; phosphatidylinositol 4,5 bisphosphate, ($PIP_2$) ; phosphatidylinositol 4 phosphate, (PIP) ; phosphatidylinositol, (PI).

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(7, 8). Since PDGF can be released from the platelet α granules we hypothesized that it could act on platelet membrane and have similar effects as phorbol esters on platelet metabolism. This work demonstrates that PDGF could play a role in platelet activation by modifying the phosphoinositide metabolism and platelet response.

## MATERIAL AND METHODS

$^{32}$P-orthophosphate (1 mCi/ml) and $^{14}$C-serotonin (50 mCi/mmole) were purchased from the Radiochemical centre (Amersham, U.K.) ; human thrombin and arachidonic acid from Sigma Chemical Co (St Louis, U.S.A.) collagen type I from Diagnostica Stago (Asnières, France).

Porcine PDGF was a gift from P. Stroobant, and was purified as in (9). The material had a purity of PDGF more than 95 %. Biological activity, monitored with a DNA synthesis assay using Swiss 3T3 cells, was estimated to 35 U/µg

Platelet suspensions were prepared as previouly described (10). Platelet rich plasma (PRP) was incubated 90 min at 37°C with $^{32}$P-orthophosphate (1 mCi/20 ml). After this incubation, the platelets were washed and centrifuged onto a metrizamide gradient. Then, platelets were resuspended in NaCl 140 mM, KCl 3 mM, $NaHCO_3$ 12 mM, glucose 10 mM, $MgCl_2$ 0.5 mM, pH = 7.4 and adjusted to 5 X $10^8$ platelets/ml.
Platelet suspension aliquots were incubated at 37°C in an aggregometer in presence of PDGF (100 ng/ml). The incubation was stopped at designated times by transferring the suspension in 1.9 ml ice cold chloroform/methanol/HCl 12 N/EDTA 0.1 M (20/40/1/2, v/v). The phospholipids were extracted and separated after centrifugation (11). The organic phase was evaporated under $N_2$ at 37°C resuspended in chloroform and applied to a cellulose thin layer chromatography plate (Merck 5716). PPI were separated with Butanol/acetic acid/$H_2O$ (150/20/50, v/v). The different phospholipids ($PIP_2$, PIP, PI) were indentified by coloration with Nil blue, scrapped and the radioactivity was quantified by liquid scintillation.

Platelets were labeled with 0.6 µM $^{14}$C-serotonin and washed as above. To avoid reuptake of released serotonin, chlorimipramine (2 µM) was added. Platelet samples (0.4 ml) were incubated in an aggregometer with a range of PDGF concentrations (25 to 200 ng/ml) before addition of saline, thrombin (0.05 U/ml), or collagen (5 to 20 µg/ml) or arachidonic acid (200 µg/ml). The reaction was stopped by transferring the suspension into 0.1 ml EDTA 0.1 M and immediatly centrifuged for 30 seconds in an Eppendorf microcentrifuge. The aggregation was stopped 3 minutes after the addition of thrombin and arachidonic acid and 5 minutes after the addition of collagen. The release of $^{14}$C-serotonin was immediately measured in a liquid scintillation spectrometer.

## RESULTS AND DISCUSSION

### Effect of PDGF on PPI metabolism

Modifications of inositol phospholipids induced by PDGF (100 ng/ml) are shown in fig. 1. PDGF progressively decreased the amount of $^{32}$P-$PIP_2$ which falled to 72 % of the initial value after 3 minutes. During the same time, the levels of $^{32}$P-labeled PIP and PI progressively increased up to respectively 120 % of the initial values. Thus, in

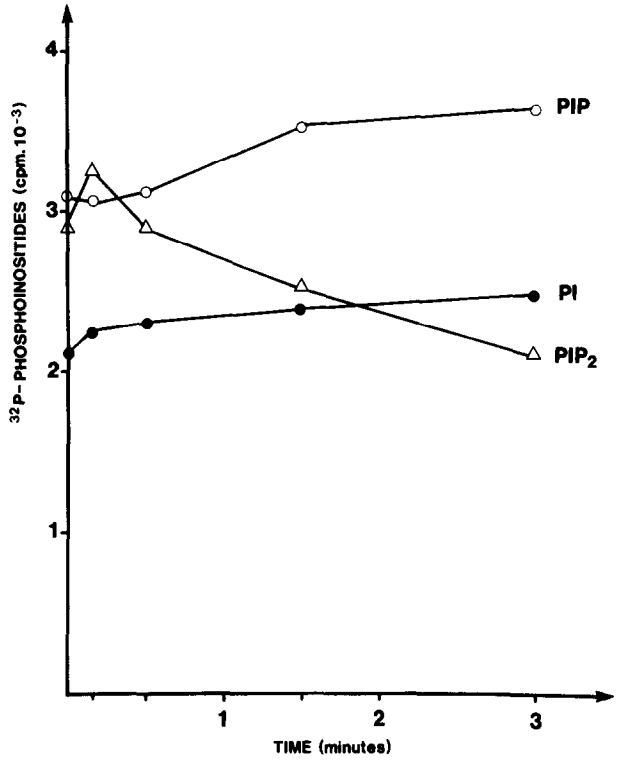


Figure 1 : Time course of PDGF effects on $^{32}$P-labelled PI, PIP, PIP$_2$. PDGF (100 ng/ml) was added to $^{32}$P-prelabelled human platelets (5 X 10$^8$ cells/ml) and incubated for different times at 37°C in the aggregometer. Representative results are from duplicate experiments performed on 4 different donors.

presence of PDGF, the decrease of the amount of $^{32}$P-PIP$_2$ was not followed by a resynthesis, but was accompanied by a similar increase in $^{32}$P-PIP and PI. This strongly suggests that the modifications induced by PDGF were rather related to either a stimulation of phosphomonoesterases or an inhibition of kinases than a stimulation of phospholipase C. Both enzymes have been reported to be associated within the plasma membrane (12, 13) and susceptible to be modulated by external stimuli. PPI changes induced by phorbol esters, recently reported i.e. increases of PIP$_2$ and PIP, differed from that of PDGF. As already suggested, phospholipase C is unlikely to be involved (7). Nevertheless it cannot be totally excluded since diacylglycerol and phosphatidic acid have not been measured in our study.

These PPI changes induced by PDGF (100 ng/ml) were not accompanied by any measurable aggregation nor platelet release ($^{14}$C-serotonin release < 1.1 %, $\beta$ thromboglobulin release < 5.4% ).

The PDGF concentration dependency of inositol phospholipid distribution after 3 minutes of incubation is shown in Fig. 2. Significant variations in $PIP_2$, PIP and PI were progressively observed with increasing concentrations of PDGF and reached a maximum with 100 ng/ml of PDGF at respectively 72 %, 110 % and 135 % of initial values. However the physiological relevance of such PDGF concentrations is difficult to extrapolate to the possible in vivo concentrations.

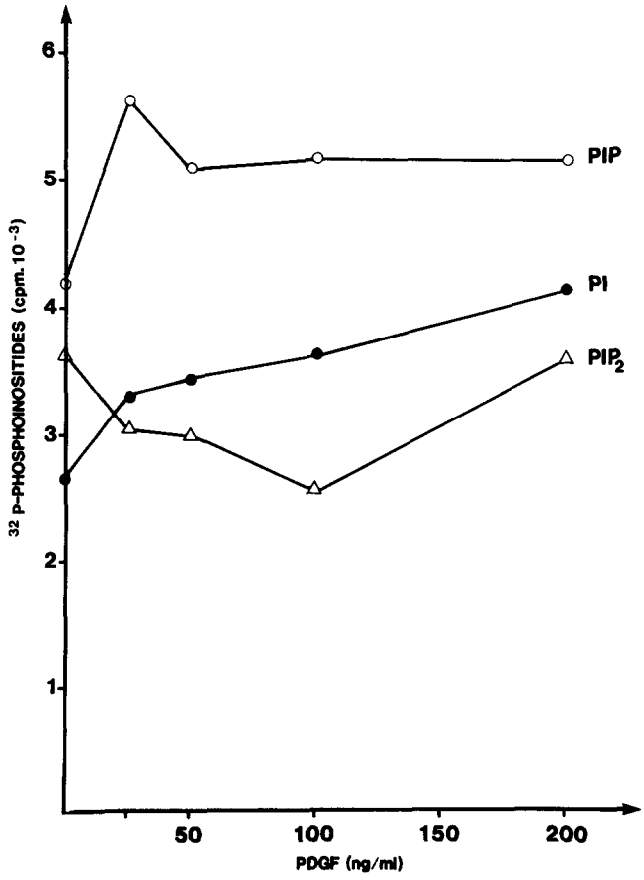


Figure 2 : Effect of various PDGF concentrations on $^{32}$P incorporation into phospholipids of human washed platelets. Platelets (5 X $10^8$ cells/ml) were incubated 3 minutes at 37°C with different concentrations of PDGF (25 ng to 200 ng/ml). Representative results from duplicate experiments performed on 3 different donors.

TABLE I : Effect of PDGF on platelet aggregation and $^{14}$C-serotonin release induced by thrombin and type I collagen

| | THROMBIN (0.05 U/ml) | | TYPE I COLLAGEN (5 to 20 µg/ml) | | |
|---|---|---|---|---|---|
| PDGF ng/ml | Aggregation I max (%) | $^{14}$C 5-HT Release (%) | Aggregation I max (%) | $^{14}$C 5-HT Release (%) | Lag phase (Time in seconds) |
| 0 | 48 +/- 7 | 54 +/- 18 | 56 +/- 8 | 45 +/- 5 | 90 |
| 25 | 44 +/- 7 | 47 +/- 10 | 45 +/- 12 | 36 +/- 9 | 90 |
| 50 | 27 +/- 9 | 29 +/- 20 | 40 +/- 3 | 36 +/- 12 | 107 |
| 100 | 12 +/- 11 | 17 +/- 21 | 23 +/- 15 | 18 +/- 4 | 120 |
| 200 | 6 +/- 6 | 11 +/- 9 | 12 +/- 10 | 15 +/- 9 | 139 |

Results are expressed in percent of maximum intensity for aggregation (I max) and of total $^{14}$C-serotonin content for release (Mean value of 3 different duplicate experiments).

Effect of PDGF on platelet aggregation and $^{14}$C-serotonin release induced by thrombin, collagen and arachidonic acid

As shown in Table I, the platelet aggregation and $^{14}$C-serotonin release induced by a low dose of thrombin (0.05 U/ml) were inhibited in presence of PDGF. The inhibition of aggregation and $^{14}$C-serotonin release was only 44 % and 47 % respectively with 50 ng/ml of PDGF (Table I). Maximal inhibition of platelet aggregation (87 %) and $^{14}$C-serotonin release (80 %) was obtained with 200 ng/ml of PDGF.

The inhibition of platelet aggregation induced by collagen is shown in Table I. A significant inhibition was observed with 100 ng/ml and 200 ng/ml of PDGF (58 % and 78 %) (Table I), with an increased lag phase (133 and 153 %). Preincubation of platelets with PDGF for 3 minutes before the addition of collagen resulted in a greater inhibition : respectively 85 % and 100 % with 100 ng/ml and 200 ng/ml of PDGF. An inhibition of $^{14}$C-serotonin release (39 % and 66 %) was also observed (Table I). Since PDGF inhibited collagen as well as thrombin aggregation and release, an inhibitory effect at the level of the platelet receptor for thrombin seemed to be excluded. PDGF (at any

concentrations from 25 to 200 ng/ml) had no effect on platelet aggregation and $^{14}C$-serotonin release induced by arachidonic acid. This suggests that arachidonic pathway is not involved in the inhibition mechanism. These results can be compared with the action of phorbol esters on human platelets, which inhibited aggregation and release of serotonin induced by thrombin and collagen but not by ionophore A 23 187 (14).

The present results seem in favour of a role for PDGF in a negative feed back control similary to the proposed action of tumor promoting phorbol myristate acetate (15). The released PDGF from $\alpha$ granules during platelet secretion, therefore, could modulate platelet response. Similarities between PDGF and phorbol esters in their ability to modify PPI metabolism within many cell types can be extrapolated to the platelets. The mechanism and the physiological relevance of PDGF - platelet interactions remain to be explained.

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