

Platelet-derived-growth-factor-induced signalling in human platelets: phosphoinositide-3-kinase-dependent inhibition of platelet activation

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Human platelets release platelet-derived growth factor (PDGF) from α -granules during platelet activation. We have previously shown that platelets have PDGF α -receptors, a transmembrane tyrosine kinase that takes part in negative feedback regulation during platelet activation. Here we have described a study of PDGF-induced tyrosine phosphorylation of platelet substrates and phosphoinositide 3-kinase (PI-3K) activity in collagen-stimulated platelets. By immunoblotting with phosphotyrosine antibodies of collagen-activated platelets we found that PDGF increased the phosphorylation of several platelet substrates, e.g. pp140, pp120 and pp85. PDGF inhibited collagen-induced platelet activation in the presence of inhibitors of autocrine stimulation, thus blocking the pure collagen-induced signal

transduction. PDGF enhanced the collagen-induced formation of PtdIns(3,4) P_2 and PtdIns(3,4,5) P_3 as measured by HPLC. Wortmannin and LY294002, two unrelated inhibitors of PI-3K, were used to investigate the role of PI-3K in PDGF-induced platelet signalling. Incubation of platelets with wortmannin and LY294002 blocked the formation of three phosphorylated inositides as well as the inhibitory effect of PDGF on collagen-induced platelet activation. We conclude that the inhibitory effect of PDGF on platelet activation is PI-3K dependent. This is the first demonstration of a negative regulatory function of 3-phosphorylated inositides in platelets.

Key words: PDGF, PI-3K.

INTRODUCTION

Platelet-derived growth factor (PDGF) is a potent mitogen and chemotactic factor for mesenchymally derived cells (reviewed in [1,2]). Receptors for PDGF are also found on other cell types, e.g. capillary endothelial cells [3], neuronal cells [4] and human platelets [5]. We have previously shown that PDGF released from the platelet α -granules takes part in negative feedback regulation of platelet activation in gel-filtered platelets [5] and in whole blood [6].

PDGF is a 30 kDa dimeric peptide that exists as homodimers and heterodimers of disulphide-linked A- and B-chains [7–9]. PDGF-BB binds to both the α - and β -receptor, whereas PDGF-AA binds only to the PDGF α -receptor. Ligand binding induces receptor dimerization and activation of the intrinsic tyrosine kinase activity of the receptor. This autophosphorylation of receptor tyrosine residues serves as docking sites for SH2-domain-containing substrate proteins or enzymes [10,11]. The PDGF receptor (PDGFR) tyrosine kinase phosphorylates and activates many of these associated SH2-domain-containing signal molecules and thereby initiates a number of signal transduction pathways that eventually result in biological responses.

Human platelets contain PDGFR- α ; known target substrates in other cell types include the phosphoinositide-specific phospholipase $C_{\gamma 1,2}$ ('PLC γ ') [12,13], the 85 kDa regulatory subunit of phosphoinositide 3-kinase (PI-3K) [14,15], the phosphotyrosine phosphatase SHP-2 [16], members of the Src family such as c-Src, c-Yes and c-Fyn [17] and the GTPase-activating protein of Ras ('GAP') [18,19].

Both PDGFR- α and - β stimulate DNA synthesis and chemotaxis. Interestingly, though, PDGFR- α has been shown to inhibit chemotaxis and Ca^{2+} release in certain cell types [20–22]. Identification of PDGFR isoform-specific signalling has been investigated by several groups; the PDGFR- α -dependent inhibitory signal for chemotaxis has been suggested to be downstream of the PI-3K pathway [23].

Here we show by immunoblotting that collagen-induced phosphorylation of several platelet proteins is augmented by PDGF. AG1296, a PDGFR- α tyrosine kinase inhibitor, blocked these effects of PDGF. Platelet activation results in the secretion and formation of several platelet agonists that enhance the platelet response (autocrine stimulation). To study the effect of PDGF on the pure collagen-induced responses we therefore incubated platelets with known inhibitors of platelet autocrine agonists. Our results demonstrate that PDGF blocks the pure collagen-induced signal and not the autocrine feedback during platelet activation.

Wortmannin and LY294002, two unrelated inhibitors of PI-3K, were used to study the role of 3-phosphorylated inositides during PDGF-induced platelet inhibition. We found that both inhibitors totally blocked collagen- and PDGF-induced PI-3K products. Moreover, the inhibitory effect of PDGF on platelet activation, measured as the formation of platelet-derived microparticles (PMPs), was abolished in the presence of wortmannin and LY294002. In the presence of inhibitors of autocrine stimulation (IAS), wortmannin or LY294002 had no effect on collagen-induced microparticle shedding itself. We conclude that the inhibitory effect of PDGF on human platelet activation is dependent on PI-3K.

Abbreviations used: CP, creatine phosphate; CPK, creatine phosphokinase; IAS, inhibitors of autocrine stimulation; PI-3K, phosphoinositide 3-kinase; PMP, platelet-derived microparticle; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; RGDS, Arg-Gly-Asp-Ser.

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MATERIALS AND METHODS

Materials

Human recombinant PDGF-BB was generously provided by Professor Carl-Henrik Heldin (Ludwig Institute for Cancer Research, Uppsala, Sweden) or obtained from Boehringer Mannheim (Mannheim, Germany). Native collagen fibrils from equine tendons were purchased from Nycomed Arzneimittel (München, Germany) and stored at 4 °C; stock solutions of bovine thrombin (Parke-Davis, Morris Plains, NJ, U.S.A.) were stored at -20 °C. Thrombin was diluted with 0.15 M NaCl to desired concentrations just before the experiments. Freeze-dried PDGF-BB (10 µg), rehydrated in 10 mM acetic acid and stored at -20 °C, was diluted with PBS (136.7 mM NaCl/2.7 mM KCl/13.1 mM Na₂HPO₄/1.5 mM KH₂PO₄) before use. Vehicle for PDGF-BB was 10 mM acetic acid diluted with PBS. Protein A-Sepharose C-4B and Sepharose CL-2B were from Pharmacia Biotech (Uppsala, Sweden). Creatine phosphate (CP), creatine phosphokinase (CPK) and cyproheptadine were obtained from Sigma (St Louis, MO, U.S.A.), SQ29.548 was from Research Biochemicals International (Natick, MA, U.S.A.), and BN 52021 (Ginkgolide-B) was obtained from Biomol Research Laboratories (Plymouth Meeting, PA, U.S.A.). Unlabelled CaliBRITE beads and FITC-labelled CaliBRITE beads were purchased from Becton Dickinson Immunocytometry (BDIS, San Jose, CA, U.S.A.). Phosphotyrosine-specific antibody 4G10 was from Upstate Biotechnology (Lake Placid, NY, U.S.A.), and the horseradish peroxidase-conjugated secondary antibody was from Bio-Rad Laboratories (Hercules, CA, U.S.A.). Enhanced chemiluminescent substrate for the detection of horseradish peroxidase was obtained from Pierce (Rockford, IL, U.S.A.). FITC-conjugated anti-(human platelet) chicken antibody was from Biopool (Umeå, Sweden). Arg-Gly-Asp-Ser (RGDS) and the PDGFR inhibitor tyrphostin AG1296 were obtained from Calbiochem-Novabiochem Corporation (San Diego, CA, U.S.A.). The PI-3K inhibitor wortmannin and LY294002 were from Sigma.

Platelet isolation

Platelet-rich plasma from fresh human blood, anticoagulated with acid citrate dextrose, was prepared as described previously [24]. In the phosphoinositide experiments, platelets were suspended in a Ca²⁺- and phosphate-free Tyrode's buffer adjusted to pH 6.7 with acid citrate dextrose before incubation with [³²P]P_i (0.5 µCi/ml) for 1 h at 37 °C. The platelets were then transferred by gel filtration into a Ca²⁺- and phosphate-free Tyrode's buffer containing 5 mM glucose and 0.2% BSA at pH 7.3. The platelet concentration, determined with a Coulter counter, was adjusted to 3.5 × 10⁸ cells/ml.

Incubations

Activation of gel-filtered platelets was performed in polypropylene tubes with the use of a Payton dual-channel aggregation module. The gel-filtered platelets were preincubated for 2 min at 37 °C in the absence or presence of five IAS that act extracellularly [the ADP removing system CP/CPK (5 mM/10 units/ml); the selective thromboxane A₂ antagonist SQ 29.548 (150 µM); RGDS (150 µM), which prevents fibrinogen from binding; the serotonin antagonist cyproheptadine (5 µM); and the platelet-activating factor antagonist, BN 52021 (150 µM)] and for 30 min with the PDGFR tyrosine kinase inhibitor tyrphostin AG1296 (5 µM). The samples were then preincubated with recombinant PDGF-BB or vehicle for 1 min with stirring (800 rev./min) at 37 °C, before incubation with thrombin or collagen.

Flow cytometric measurements of shedding of PMPs

Flow cytometric detection of PMP formation was performed as described previously [6]. In brief, aliquots were fixed with 19 vol. of 0.2% paraformaldehyde in PBS (1:19, v/v) and then incubated with a saturating concentration of FITC-conjugated anti-(human platelet surface) chicken antibody. Gating on both FITC and light-scatter profiles identified PMPs [6].

Immunoblot analysis

Detection of phosphotyrosine-containing proteins was performed in accordance with the manufacturer's instructions (Upstate Biotechnology, Lake Placid, NY, U.S.A.). In brief, platelet lysates in SDS-containing lysis buffer [280 mM Tris/HCl (pH 7.2)/40% (v/v) glycerol/0.01% Bromophenol Blue/8% (w/v) SDS/4% (v/v) 2-mercaptoethanol/4 mM orthovanadate] were separated by SDS/PAGE [5–15% (w/v) gradient gel] under reducing conditions and blotted on a 0.2 µm pore-size nitrocellulose filter (Schleicher and Schuell, Dassel, Germany). The nitrocellulose was rinsed twice for 5 min in distilled water before it was incubated for 2 h in blocking solution {3% (w/v) dried non-fat milk (Nestlé) in TBST [250 mM Tris/HCl (pH 8)/100 mM NaCl/0.01% Tween-20]}. Immunoblots were probed overnight with the phosphotyrosine-specific antibody 4G10 in blocking solution. The nitrocellulose was washed twice with water and then incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (Bio-Rad) in blocking solution. Finally, the immunoreactivity of the tyrosine-phosphorylated proteins was detected with the enhanced chemiluminescence reaction (Pierce, Rockford, IL, U.S.A.).

Extraction and analysis of 3-phosphorylated glycerophosphoinositides

Extraction and TLC of polyphosphoinositides from platelets were performed as described elsewhere [24]. In principle, aliquots of platelet incubation mixtures were mixed with methanol/chloroform/HCl (20:40:1, by vol.); phase separation was induced by adding appropriate quantities of chloroform and water and the mixture was then centrifuged at 210 g for 3 min. The organic phase was concentrated with a jet of argon before the radiolabelled phospholipids were separated on silica plates (silica gel 60; Merck, Darmstadt, Germany). Radioactivity was detected with an Instant Imager radioactivity scanner (Packard Instrument Company, Meriden, CT, U.S.A.); spots containing radiolabelled PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃ were scraped off the plates. The silica powder was heated with 1 ml of deacylating reagent [26.8 ml of methylamine (40% in water)/45.7 ml of methanol/11.4 ml of butan-1-ol/16.0 ml of water] at 53 °C for 50 min before being dried under an argon stream. The powder was then dissolved in 1.2 ml of water, centrifuged and finally filtered through a Millipore HV 0.45 µm pore-size filter; the filtrate was subjected to HPLC, being eluted with a 0–2 M (NH₄)₃PO₄ (pH 3.8) gradient by the method of Auger et al. [14]. The radioactive peaks were detected in the eluate by Cerenkov scintillation with a flow-radioactive monitor (Ramona D; Raytest, Münster, Germany). The output from the monitor was recorded online with a standard PC using a computer program (µCrom) developed by Daniel Cejka.

Statistics

Results are presented as means ± S.E.M. The statistical significance was determined by paired-sample Student's *t* test.

RESULTS

Collagen-induced tyrosine phosphorylation of platelet proteins is augmented by PDGF

We have previously demonstrated that human platelets have functionally active PDGF α -receptors and that an autocrine inhibition pathway is transduced through this tyrosine kinase

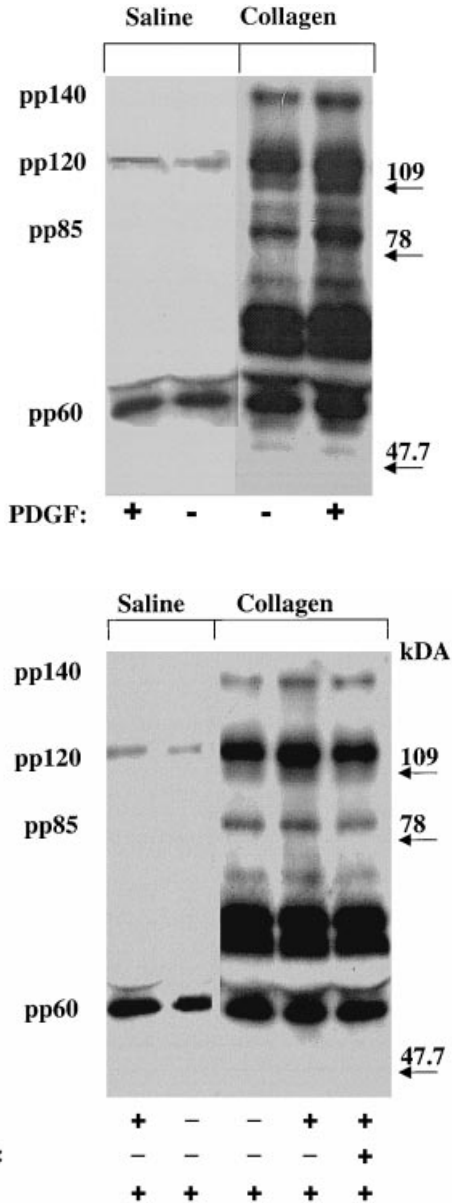


Figure 1 Collagen-induced phosphorylation of platelet proteins is augmented by PDGF

Platelets were preincubated for 2 min at 37 °C without (upper panel) or with (lower panel) IAS; CP/CPK (5 mM/10 units/ml)/SQ 29.548 (150 μ M)/RGDS (150 μ M)/cyproheptadine (5 μ M)/BN 52021 (150 μ M) and for 30 min with the PDGFR tyrosine kinase inhibitor tyrphostin AG1296 (5 μ M). The samples were stirred with PDGF BB (100 ng/ml) or vehicle for 1 min (800 rev./min) before incubation with collagen (50 μ g/ml) for 1 min at 37 °C. Platelet lysates were separated by SDS/PAGE [5–15% (w/v) gradient gel] under reducing conditions and blotted to nitrocellulose paper. Tyrosine-phosphorylated proteins were probed with mAb 4G10 and detected by enhanced chemiluminescence as described in the Materials and methods section. The Figure shows one representative immunoblot of six independent experiments. The positions of molecular mass markers are indicated (in kDa) at the right.

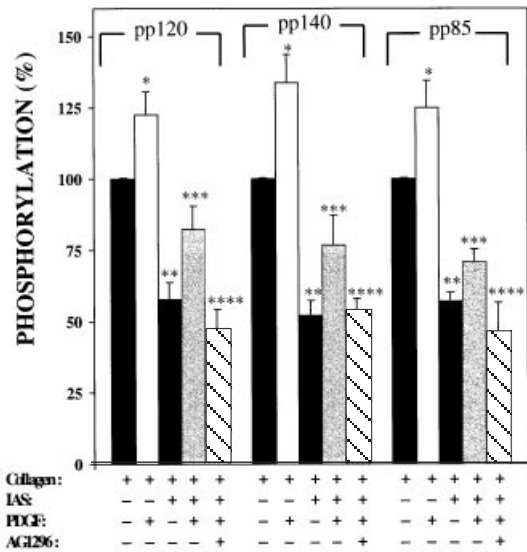


Figure 2 Quantification of protein components in platelets treated with agonist and antagonist

The relative amount of phosphotyrosine in the pp140, pp120 and pp85 components in experiments illustrated in Figure 1 were quantified by densitometric scanning of the immunoblot. The amounts of phosphorylation in the presence of collagen were set at 100%. Results are means \pm S.E.M. for six different experiments. Statistical comparisons were determined by a paired-sample Student's *t* test. **P* < 0.05 for collagen + vehicle compared with collagen + PDGF; ***P* < 0.05 for collagen + vehicle compared with collagen + IAS; ****P* < 0.05 for collagen + IAS compared with collagen + PDGF + IAS; *****P* < 0.05 for collagen + IAS + PDGF compared with collagen + IAS + PDGF + tyrphostin AG1296.

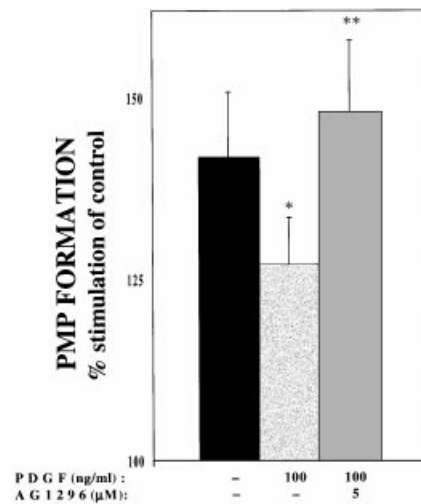


Figure 3 PDGF inhibits collagen-induced PMP formation in the presence of IAS

Platelets were preincubated with or without IAS, AG1296 and PDGF before activation with collagen (50 μ g/ml) as described in the legend to Figure 1. Aliquots of gel-filtered platelets were fixed with 19 vol. of 0.2% paraformaldehyde and then incubated with a saturating concentration of FITC-conjugated anti-(human platelet) chicken antibodies. PMP formation was detected in a FACSsort flow cytometer as described in the Materials and methods section. The amount of PMP formation in the absence of collagen was set at 100%. Results are means \pm S.E.M. for six different experiments. Statistical comparisons were determined by a paired-sample Student's *t* test. **P* < 0.05 for collagen + IAS compared with PDGF + collagen + IAS; ***P* < 0.05 for PDGF + collagen + IAS compared with PDGF + collagen + IAS + AG1296.

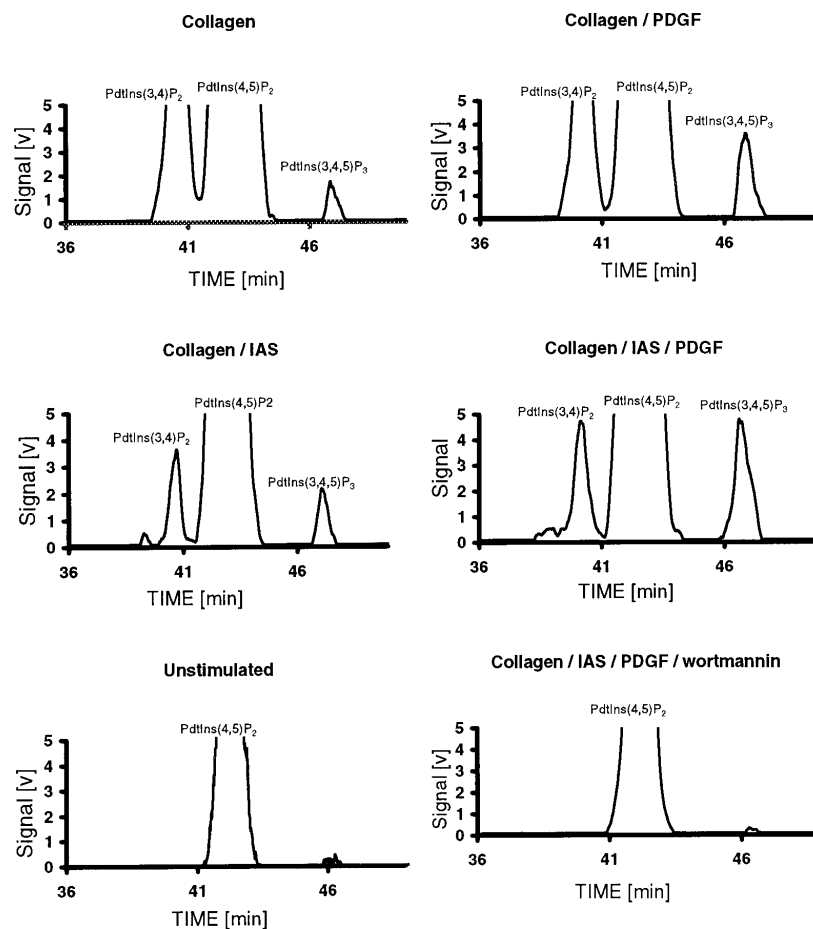


Figure 4 Resolution of 3-phosphorylated polyphosphoinositides by HPLC

³²P-labelled platelets were preincubated without or with PDGF (250 ng/ml), IAS and wortmannin (100 nM) before activation with collagen (50 μ g/ml) as described in the legend to Figure 1. Radiolabelled deacylated phospholipids (glycerophosphoinositols) were analysed by HPLC as described in the Materials and methods section.

receptor during platelet activation [5]. In view of these results we explored platelet substrates potentially modified by PDGF during stimulation with collagen. Platelets were stimulated with collagen (50 μ g/ml) and/or PDGF (100 ng/ml) before SDS/PAGE and immunoblotting with anti-phosphotyrosine antibody. As shown in Figure 1 (upper panel), collagen alone resulted in increased tyrosine phosphorylation of several protein bands. PDGF significantly enhanced the collagen-induced phosphorylation of several components, for example proteins of 140, 120 and 85 kDa. As observed previously [5,25], no effect of PDGF was found in unstimulated platelets. Studies have shown that specific binding of PDGF to platelets is dependent on platelet activation [25]. Thus PDGF modulates several platelet substrates during activation by collagen.

Platelet substrate phosphorylation in the presence of IAS

Platelet activation by collagen results in the secretion and synthesis of several platelet agonists from platelet granules that enhance the platelet response, the so-called positive feedback or autocrine stimulation. Thus the previously reported platelet inhibitory effect of PDGF on thrombin- and collagen-induced responses might be due to modulation of the primary ligand

signal or to interference with one or several steps in the positive feedback regulation [5,25,26]. A cocktail of five IAS, containing CP/CPK (which removes ADP), SQ 29,548 (a thromboxane A₂ antagonist), RGDS (which prevents fibrinogen from binding), cyproheptadine (a serotonin antagonist) and BN 52021 (a platelet-activating factor antagonist) has previously been shown to block autocrine platelet activation efficiently [27]. Therefore, to study the effect of PDGF on the pure collagen-induced responses only, we preincubated platelets with or without IAS. Platelets were then stimulated with collagen (50 μ g/ml) and/or PDGF (100 ng/ml) before SDS/PAGE and immunoblotting with anti-phosphotyrosine antibody.

As shown in Figure 1 (lower panel), collagen alone resulted in the increased tyrosine phosphorylation of several protein bands in the presence of IAS too. This effect of collagen was enhanced by PDGF. Thus PDGF seemed to modulate the pure collagen-induced signal transduction. To ensure that this effect was dependent on PDGF, control cells were also incubated with the PDGFR antagonist tyrphostin AG1296. AG1296 clearly inhibited the effect of PDGF (Figure 1, lower panel). The relative tyrosine phosphorylations of the 140, 120 and 85 kDa components were measured by densitometric analysis of the immunoblots and are shown in Figure 2.

Table 1 Collagen-induced formation of 3-phosphorylated polyphosphoinositides is augmented by PDGF

³²P-labelled platelets were incubated with collagen (50 µg/ml) for 5 min and/or PDGF (250 ng/ml) and IAS as described in the legend to Figure 1. Radiolabelled deacylated phospholipids (glycerophosphoinositides) were analysed by HPLC as described in the Materials and methods section. Results are means ± S.E.M. for three separate experiments. The collagen-induced formation of PtdIns(3,4)P₂ (15630 c.p.m.) and PtdIns(3,4,5)P₃ (2750 c.p.m.) in the absence of PDGF and IAS was set at 100%.

Product	Phosphorylation (%)			
	Collagen	Collagen/PDGF	Collagen/IAS	Collagen/PDGF/IAS
PtdIns(3,4)P ₂	100 ± 28	238 ± 89	54 ± 4	104 ± 40
PtdIns(3,4,5)P ₃	100 ± 20	171 ± 35	143 ± 64	309 ± 130

PDGF inhibits collagen-induced platelet activation in the presence of IAS

The effect of PDGF on collagen-induced tyrosine phosphorylation was also complemented by measurements of flow cytometric platelet activation parameters. In parallel with the immunoblot analysis above, platelets were pretreated with IAS and incubated with collagen (50 µg/ml) and/or PDGF (100 ng/ml) and AG1296. Samples were then analysed by flow cytometry for the shedding of procoagulant PMPs from the platelet plasma membrane during platelet activation. As shown in Figure 3, PDGF significantly inhibited collagen-induced PMP formation in the presence of IAS; similar results were obtained by measuring platelet aggregation (results not shown). Thus the effect of PDGF on platelet tyrosine phosphorylation in the presence of IAS, as demonstrated in Figure 1, was paralleled by inhibition of platelet activation (Figure 3).

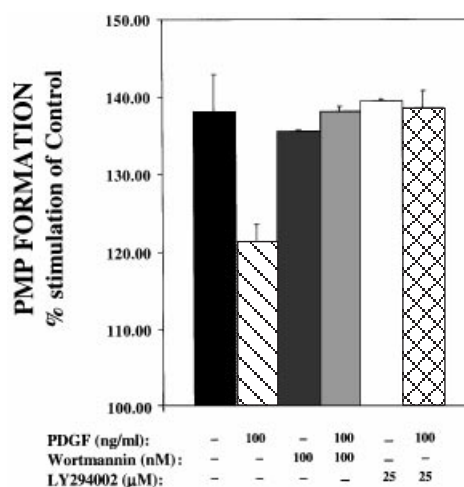
Platelets were also preincubated with the PDGFR tyrosine kinase inhibitor tyrphostin AG1296, which potentiated collagen-induced PMP formation (Figure 3). Thus AG1296 seems to block the inhibitory signalling transduced through PDGFR-α during platelet activation.

PI-3K dependent inhibition of platelet activation by PDGF

As shown in Figure 1, PDGF significantly increased the phosphorylation of a protein component corresponding to p85. PDGFR-associated PI-3K activity has been shown to be highly susceptible to inhibition by AG1296 in pig aortic endothelial cells [28]. Moreover, the inhibitory effect of PDGFR-α on PDGFR-β-induced chemotaxis was suggested to be downstream of PI-3K [23]. We therefore decided to study the role of PI-3K on the PDGF-induced responses in platelets with the use of wortmannin and LY294002, two unrelated inhibitors of PI-3K [29]. Platelets were prelabelled with [³²P]P_i and incubated with collagen (50 µg/ml) and/or PDGF (250 ng/ml) and wortmannin (100 nM) in the absence or presence of IAS. Parallel samples were studied by TLC and HPLC of radiolabelled 3-phosphorylated polyphosphoinositides (Figure 4 and Table 1) and by flow cytometry for platelet activation (Figure 5).

As shown in Figure 4 and Table 1, incubation of platelets with collagen induced the formation of PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃, in both the absence and the presence of IAS. PDGF increased the collagen-induced phosphorylation of the 3-phosphorylated phosphoinositides (Table 1).

The addition of wortmannin (100 nM) abolished the formation of 3-phosphorylated phosphoinositides to undetectable levels, whereas the formation of PtdIns(4,5)P₂ was unaffected (Figure

**Figure 5** Blocking of PI-3K activity by wortmannin abrogates the inhibitory effect of PDGF on PMP formation

Platelets were preincubated with or without PDGF (100 ng/ml), wortmannin (100 nM), LY294002 (25 µM) and IAS before activation with collagen (50 µg/ml) as described in the legend to Figure 1. PMP formation was detected in a FACSort flow cytometer as described in the legend to Figure 3. The amount of PMP formation in the absence of collagen was set at 100%. The figure shows one of four different experiment performed in triplicate; means ± S.E.M.

4). Thus the collagen-induced formation of 3-phosphorylated phosphoinositides was enhanced by PDGF and completely inhibited by wortmannin. Similar results were obtained with the PI-3K inhibitor LY294002 (results not shown). PDGF alone had no effect on the 3-phosphorylated phosphoinositides in resting platelets.

Parallel samples were studied by flow cytometry for platelet activation (Figure 5). In the presence of IAS, collagen increased the PMP formation. This effect of collagen was inhibited by PDGF. Interestingly, the addition of wortmannin (100 nM) or LY294002 (25 µM) did not significantly affect the collagen-induced platelet activation in the presence of IAS. Furthermore, the inhibitory effect of PDGF on collagen-induced platelet activation was completely blocked by wortmannin and LY294002 (25 µM) (Figure 5). We therefore conclude that the inhibitory effect of PDGF on collagen-induced platelet activation is entirely dependent on PI-3K.

DISCUSSION

The observed inhibitory effect of PDGF on collagen-induced or thrombin-induced platelet activation might be due to modulation of the pure ligand signal or to interference with one or several steps in the positive feedback regulation. Thrombin-induced protein tyrosine phosphorylation was recently found to be entirely due to autocrine stimulation, whereas phosphorylation in response to collagen is due to collagen itself [27]. The use of IAS that block ADP, thromboxane, serotonin, platelet-activating factor and fibrinogen allowed us to study the effect of PDGF on pure collagen-induced signalling. We found a parallel effect of PDGF on substrate tyrosine phosphorylation and inhibition of platelet activation by collagen. Thus PDGF seems to block the isolated collagen-induced signal and not the autocrine feedback during platelet activation.

We have recently shown that collagen, in contrast with thrombin, induced the formation of PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃ in both the absence and the presence of IAS; thus

collagen by itself increases the level of PtdIns(3,4) P_2 and PtdIns(3,4,5) P_3 . We show that an 85 kDa component corresponding to the regulatory unit of PI-3K is phosphorylated by PDGF during collagen stimulation (Figure 2). Furthermore, PDGF enhanced the collagen-induced phosphorylation of PtdIns(3,4) P_2 and PtdIns(3,4,5) P_3 in both the absence and the presence of IAS. We found that wortmannin and LY294002, two unrelated inhibitors of PI-3K, totally blocked collagen-induced and PDGF-induced PI-3K products. Interestingly, in parallel experiments the inhibitory effect of PDGF on platelet activation, measured as PMP formation, was abolished in the presence of wortmannin and LY294002.

In agreement with our results, PI-3K (pp85) has recently been shown to be a substrate for a protein tyrosine kinase in human platelets [30]. The role of PI-3K in human platelet activation is still unclear. Studies indicate that PI-3K contributes to integrin activation [31,32]. Studies with wortmannin have shown that PI-3K is necessary for the prolonged activation of glycoprotein GPIIb-IIIa and irreversible platelet activation, whereas platelet secretion was not affected [31]. Our results show that wortmannin blocked the formation of PtdIns(3,4) P_2 and PtdIns(3,4,5) P_3 but did not affect collagen-induced platelet activation in the presence of IAS. The IAS mixture contained RGDS, which blocks fibrinogen binding. This result is therefore in agreement with a functional role of PI-3K in platelet activation that is dependent on specific autocrine stimulation by integrin activation.

Possible targets for the inhibitory effect of PI-3K in human platelets remain to be identified. Because PI-3K activity is generally recognized to participate in activating pathways in platelets, it is possible that PDGFR has a unique and yet unknown inhibitory signalling effect involving cross-talk dependent on 3-phosphorylated inositides or on some of their downstream targets. Downstream targets of PI-3K include several possibilities such as Akt/protein kinase B ('PKB') and isoforms of phosphoinositide-specific phospholipase C ('PKC'). Interestingly, PDGF-induced inhibition of apoptosis has been suggested to be PI-3K dependent and mediated through the Akt/PKB pathway [33–35]. Negative signalling through the PI-3K system has also been reported in T-lymphocyte regulation [36,37]. These reports suggest that the products of PI-3K are important mediators of negative signalling pathways in T-cell activation and lymphocyte homeostasis and that the net effect of PI-3K activation is determined by the numbers of different receptors that simultaneously engage PI-3K.

We conclude that the PDGFR- α tyrosine kinase contributes to platelet tyrosine phosphorylation. The inhibitory effect of PDGF in human platelets involves collagen-induced signalling, which is independent of autocrine platelet activation. We found that the inhibitory effect of PDGF on platelet activation is dependent on PI-3K; this is the first demonstration of a negative regulatory function of 3-phosphorylated inositides in platelets. Thus PI-3K activity might be involved in both activatory and inhibitory signals during platelet activation.

We thank Alexander Staeffler and Daniel Cejka for technical advice on software, and Philip Anthonisen for technical assistance with the HPLC. This study was supported by grants from Norwegian National Health Association and from Biomed 2 Programme, contract no. BMH4-CT-97 2609.

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Received 23 February 2000/15 May 2000; accepted 21 June 2000