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Effect of the different dimeric forms of the platelet-derived growth factor on cellular responses in mouse Swiss 3T3 fibroblasts

E. Sturani, R. Brambilla, L. Morello, M.G. Cattaneo*, R. Zippel and L. Alberghina

Department of Biochemistry and General Physiology, Via Celoria 26 and **Department of Pharmacology, Via Vanvitelli 32, 20133 Milano, Italy*

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PDGF consists of two polypeptide chains, A and B, and all three possible dimers have been isolated from different sources. Human PDGF, essentially AB, porcine PDGF (BB) and recombinant PDGF-AA were tested on Swiss 3T3 fibroblasts for their ability to stimulate mitogenesis, phosphoinositide turnover and tyrosine phosphorylation of the PDGF receptor. When used in saturating amounts, the three isoforms were equally active in inducing mitogenesis. However, PDGF-AA was less active than AB and BB to induce the phosphorylation of the receptor and the turnover of phosphoinositides (30% and 50%, respectively). These findings suggest that, in Swiss 3T3 fibroblasts, PDGF receptors of the α-type are present in a slightly lower amount than β-type. In addition, the two types of receptor appear to have similar physiological functions.

Platelet-derived growth factor isoform; Tyrosine phosphorylation; Platelet-derived growth factor receptor; Mitogenesis; Phosphoinositide; (Mouse fibroblast)

1. INTRODUCTION

The platelet-derived growth factor (PDGF), a major mitogen for connective cells in vitro [1], structurally consists of two polypeptide chains linked by disulphide bonds [2]. The A and B subunits share 60% homology and the B chain is the product of the c-*sis* gene, the normal counterpart of the v-*sis* oncogene carried by the simian sarcoma virus [3]. All three possible dimeric forms of PDGF have been isolated from different sources: PDGF purified from human platelets (hPDGF) mainly consists of the AB heterodimer [4], porcine PDGF (pPDGF) is a BB homodimer [5], as is the transforming product of the simian sarcoma virus. PDGF-AA is produced by different human tumoral cell lines [6,7]. Two types of highly homologous PDGF receptors have been identified and cloned [8–13]: an α (or A)-type receptor which interacts with all three isoforms [12] and a β (or B)-type receptor, which interacts with BB and AB dimers according to several authors [8,11,12] or only with BB according to other groups [9,10]. The two receptor types are structurally similar and are endowed with a protein tyrosine kinase domain [12,13]. In human fibroblasts, the two receptor classes appear to be expressed in different amounts [9] and whereas PDGF-AB and BB are strongly mitogenic, PDGF-AA is poorly active [8]. In mouse fibroblasts, the situation is not very well established although it seems different from human cells [12,14,15] and it will be investigated further in this paper.

In addition to the tyrosine kinase activity of the receptors, PDGF also stimulates, in several responsive cells, phosphoinositide breakdown [16–19] and, as shown by Escobedo and Williams [20], a single type of receptor (B or β) is able to transduce the PDGF signal through both tyrosine kinase and phosphoinositide turnover pathways.

We have investigated in mouse Swiss 3T3 fibroblasts the effect of the different isoforms on mitogenesis, tyrosine phosphorylation of PDGF receptor and turnover of phosphoinositides, in order to

Correspondence address: E. Sturani, Department of General Physiology and Biochemistry, University of Milan, Via Celoria 26, 20133 Milan, Italy

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understand how the two receptor types are expressed in this cell line and to gain information about their physiological functions.

2. MATERIALS AND METHODS

2.1. *Cells and growth conditions*

Mouse Swiss 3T3 fibroblasts were grown as previously described [21]. Quiescent cells were obtained by shifting monolayers to DMEM containing 1% fetal calf serum (FCS) for 24 h.

2.2. *Thymidine incorporation*

DNA synthesis was determined according to Ross et al. [22]. Quiescent cells were stimulated for 16 h and labelled for 3 h with [^{3}H]thymidine (2.5 μCi/ml). TCA insoluble radioactivity was determined.

2.3. *Tyrosine phosphorylation*

Quiescent monolayers in 35 mm Petri dishes were incubated in 0.5 ml of binding medium [23] containing 0.5% BSA for 8 min at 37°C with the different PDGF isoforms. Total cell proteins were extracted and analyzed by immunoblotting with anti-phosphotyrosine antibodies and ^{125}I-protein A as described [21]. After autoradiography the relative band intensity was determined either by counting the ^{125}I radioactivity associated with the receptor band or by densitometric scanning.

2.4. *Phosphoinositide turnover*

Confluent monolayers were incubated with [*myo*-^{3}H]inositol (2 μCi/ml) for 24 h in an inositol-free basal Eagle medium containing 1% FCS. Cells were then stimulated with PDGF in 0.5 ml of a Krebs Ringer Hepes solution, with 0.1% BSA, containing 10 mM LiCl, for 10 min. The cells were extracted with TCA and [^{3}H]inositol phosphates (InsPs) were separated by anion-exchange chromatography [16,17].

2.5. *PDGF*

hPDGF was purified from human platelets by chromatography on carboxymethyl cellulose, Blue Sepharose, Biogel P-150 and reverse-phase HPLC [24]. The final preparation analyzed by SDS-PAGE under non-reducing conditions followed by silver staining revealed a unique band of 28–33 kDa. The PDGF-AA homodimer, purified from the conditioned medium of yeast cells transfected with PDGF A chain cDNA, was a kind gift from Dr C.H. Heldin [8]. Highly purified pPDGF (90% pure) was purchased from Bioprocessing.

2.6. *Phosphotyrosine antibodies*

Antibodies against azobenzylphosphonate, which specifically crossreact with phosphotyrosine, prepared and affinity-purified as described [25] were a kind gift from Dr P.M. Comoglio.

3. RESULTS AND DISCUSSION

The dose-dependent effects of PDGF purified from human platelets, which mainly consists of the AB heterodimer [4], pPDGF (BB) and recombinant PDGF-AA were tested on mitogenesis, phosphoinositide turnover and tyrosine phosphorylation of the PDGF receptor in Swiss 3T3 fibroblasts. Mitogenesis was tested as [^{3}H]thymidine incorporation; phosphoinositide turnover as the accumulation of total InsPs ($InsP_1$ + $InsP_2$ + $InsP_3$) during 10 min of stimulation in the presence of LiCl. Phosphorylation of PDGF receptors was determined by immunoblot analysis with phosphotyrosine antibodies in cells stimulated with PDGF for 8 min. An example of a dose response experiment on the receptor phosphorylation induced by pPDGF is reported in fig.1; the phosphorylated form of the receptor is evident in the autoradiography as a 170 kDa band and the band intensity is a function of the PDGF concentration.

For the three responses the data were calculated as the difference between the values obtained on stimulated cells and the basal value (unstimulated cells), and were expressed as a percentage of the maximal one. This made it possible to compare the effects of a single isoform on the three different physiological responses.

In the upper panel of fig.2, the results obtained with hPDGF are reported. The ED_{50} for mitogenesis is 8 ng/ml whereas the maximal response is achieved at about 25 ng/ml. For InsPs accumula-

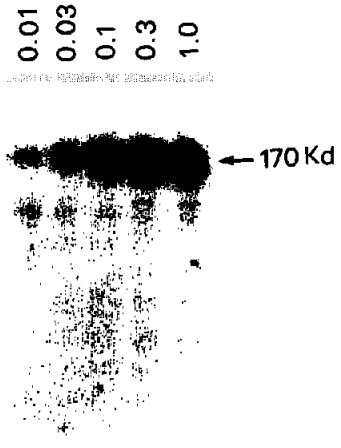

Fig.1. PDGF-induced tyrosine phosphorylation of the PDGF receptor. Swiss 3T3 fibroblasts were stimulated for 8 min with 10, 30, 100, 300 and 1000 ng/ml of pPDGF. P-tyr containing proteins were analyzed with P-tyr antibodies as reported in section 2. The data were quantitated and reported in the middle panel of fig.2.

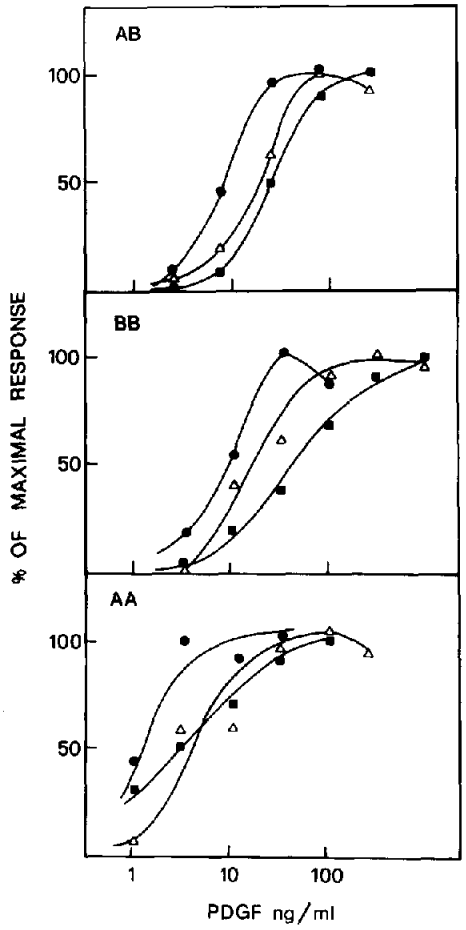

Fig.2. Dose-dependent effects of the three isoforms of PDGF on DNA synthesis, InsPs accumulation and tyrosine phosphorylation of the PDGF receptor. Quiescent Swiss 3T3 fibroblasts were stimulated with different concentrations of hPDGF (AB, upper panel), with pPDGF (BB, middle panel) and with recombinant PDGF-AA. Mitogenic activity (●), InsPs accumulation (△) and tyrosine phosphorylation of the PDGF receptor (■) were tested as reported in section 2. For each kind of measurement the activity of unstimulated cultures (basal) was subtracted from that of the stimulated ones and the data are expressed as a percentage of the maximal value. For InsPs accumulation and mitogenesis the data are the average of three independent determinations.

tion and receptor phosphorylation the dose-response curves are shifted to the right with half-maximal responses at 18 and 27 ng/ml, respectively.

The behaviour observed with pPDGF (middle panel of fig.2) is similar, the ED_{50} being 9, 18 and 50 ng/ml for the three responses. Moreover, in these cells also PDGF-AA was able to elicit the same responses: as shown in the lower panel of fig.2, the general pattern is similar to that observed with the other two dimers, but the ED_{50} values are lower (1.5, 6 and 5 ng/ml).

Thus, in Swiss 3T3 fibroblasts the three isoforms stimulate mitogenesis, phosphoinositide turnover and receptor phosphorylation. In every case, the dose-effect curve for mitogenesis was shifted toward lower concentrations with respect to the other two responses. This observation can be explained by the major differences in the protocols that have to be used for the three assays: determination of DNA synthesis involves a 16 h exposure to PDGF, whereas for the two other responses, cells are stimulated with PDGF for only 10 and 8 min. Moreover, for mitogenesis not only the concentration of the ligand but also the total amount of PDGF present in the assay may be critical.

In addition, these data indicate that a maximal stimulation of mitogenesis can be obtained even at PDGF concentrations that do not allow maximal stimulation of phosphoinositide turnover and of receptor phosphorylation.

Whereas the concentrations of human and porcine PDGF which gave half-maximal stimulation were very close, but that found for PDGF-AA was

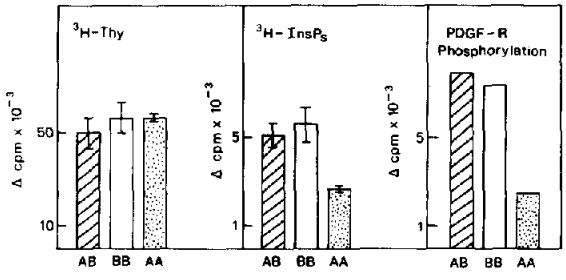

Fig.3. Effect of saturating concentrations of the three isoforms on thymidine incorporation, phosphoinositide hydrolysis and PDGF receptor phosphorylation. [^{3}H]thymidine incorporation (left panel): cells were stimulated with 50 ng/ml of PDGF AB, 50 ng/ml BB and 30 ng/ml AA. Incorporation of unstimulated cells was 1095 ± 240 cpm. InsPs accumulation (central panel) and PDGF receptor phosphorylation (right panel): cells were stimulated with 250 ng/ml of PDGF-AB, 500 ng/ml BB and 100 ng/ml AA. For InsPs accumulation basal value was 932 ± 122. The data for receptor phosphorylation were quantitated from fig.4 and are the average of two determinations. For mitogenesis and InsPs accumulation the data are the average of three determinations and standard deviations are indicated. The data are expressed as Δcpm ove the basal.

lower. This difference may be due to differences in the degree of purity of the three preparations or to possible difficulties in the determination of the real concentration of the factors, due to the fact that the three factors come from different sources.

The determination of the dose-response curves made it possible to compare the effects of the three isoforms when present at saturating concentrations: the left panel of fig.3 shows that at these concentrations the human PDGF, porcine PDGF and PDGF-AA induce the same mitogenic response, also equivalent to that determined in cells stimulated with 10% serum (not shown). Instead, in the case of phosphoinositide turnover (central panel of fig.3), the response induced by AA is about 50% of those of AB and BB (statistical analysis of the data indicated that the difference between AA and BB and the difference between AA and AB were highly significant).

Tyrosine phosphorylation of PDGF receptors induced by the three isoforms is shown in fig.4 and quantitated in the right panel of fig.3. It is evident that also in this case PDGF-AA is less effective (about 30%) than the two other forms. Fig.4 also shows that the bands phosphorylated in response to AA, AB and BB have the same apparent molecular weight, although minor differences would not have been detected.

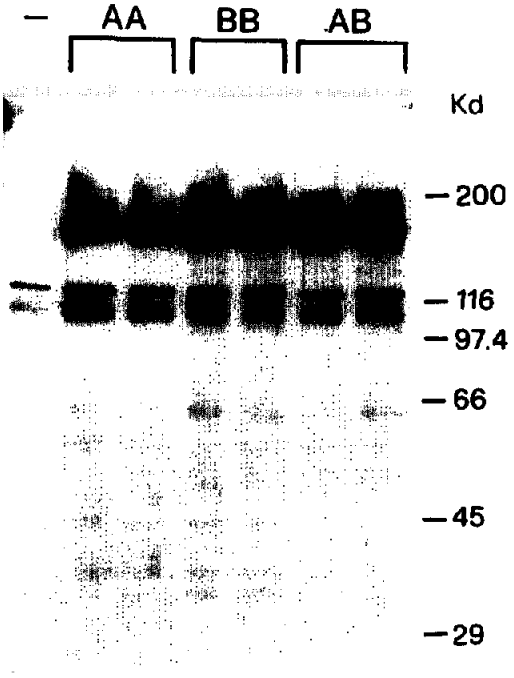

Fig.4. Tyrosine phosphorylation of PDGF receptor induced by saturating concentrations of the three different isoforms. Cells received no addition, or PDGF-AA (100 ng/ml) or PDGF-BB (500 ng/ml) or PDGF-AB (250 ng/ml).

Activation of the receptor kinase and its autophosphorylation occur at the receptor level; accumulation of InsPs, which is also a short-term response, is mediated by other transducing element(s) in addition to the receptors; instead DNA synthesis is the result of a long series of cascade events during which amplification may occur at different levels as may be suggested by the finding that the ligand concentration which gives half maximal stimulation for DNA synthesis is lower than ED_{50} for the other two responses. Whereas the responses induced by PDGF-AA are exclusively mediated by the α-type receptors, those induced by PDGF-BB are mediated by both receptor types. It is still debated whether the AB heterodimer interacts with both receptors as some authors report [8,11,12] or whether it only interacts with the α-type [9,10]. PDGF purified from human platelets contains a certain amount of PDGF-BB, in addition to the heterodimer [4] and thus we cannot distinguish between these two possibilities. However, what is clearly indicated by our data is that in Swiss 3T3 fibroblast α-type receptors are present in sufficient amounts to elicit the same mitogenic response as that observed when also β-type receptors are activated. On the other hand, stimulation of immediate events such as receptor phosphorylation and phospoinositide turnover, elicited by activation of both (α and β) is at least double compared to that mediated by α-receptors alone. This suggests that α-receptors are present in a slightly smaller amount than β-type in Swiss 3T3 fibroblasts, in accordance with the data of Kazlauskas [14].

This situation appears to be different from that observed in human fibroblasts, in which the AA form is poorly active [8] and α-receptors are 10–15% of the totals [9].

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