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Binding of different dimeric forms of PDGF to human fibroblasts: evidence for two separate receptor types

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The binding of the three dimeric forms of platelet-derived growth factor (PDGF), PDGF-AA, PDGF-AB and PDGF-BB, to human fibroblasts was studied. Cross-competition experiments revealed the existence of two different PDGF receptor classes: the type A PDGF receptor bound all three dimeric forms of PDGF, whereas the type B PDGF receptor bound PDGF-BB with high affinity and PDGF-AB with lower affinity, but not PDGF-AA. The sizes of the two receptors were estimated with affinity labeling techniques; the A type receptor appeared as a major component of 125 kd and a minor of 160 kd, and the B type receptor as two components of 160 and 175 kd. A previously established PDGF receptor monoclonal antibody, PDGFR-B2, was shown to react with the B type receptor only. The different abilities of the three dimeric forms of PDGF to stimulate incorporation of [^{3}H]TdR into human fibroblasts indicated that the major mitogenic effect of PDGF is mediated via the B type receptor.

Key words: platelet-derived growth factor/receptor/human fibroblasts

Introduction

Platelet-derived growth factor (PDGF) is a major mitogen for connective tissue cells *in vitro* (reviewed in Heldin *et al.*, 1985; Ross *et al.*, 1986). The functionally active molecule is made up as a dimer of two polypeptide chains that are linked by disulphide bonds. The subunit chains occur in two different forms, denoted A and B, and their mature parts show 60% amino acid sequence similarity with perfect conservation of all eight cysteine residues (Johnsson *et al.*, 1984; Josephs *et al.*, 1984; Betsholtz *et al.*, 1986).

All three possible dimeric forms of PDGF have been identified: PDGF purified from human platelets is a heterodimer, PDGF-AB (Hammacher *et al.*, 1988a); PDGF-AA has been found in media conditioned by human osteosarcoma (Heldin *et al.*, 1986), melanoma (Westermark *et al.*, 1986) and glioma (Hammacher *et al.*, 1988b) cell lines, and is probably produced by rat smooth muscle cells (Sejersen *et al.*, 1986) and mitogen-stimulated human fibroblasts (Paulsson *et al.*, 1987); a factor similar to PDGF-BB exerts the transforming activity of simian sarcoma virus (Waterfield *et al.*, 1983; Doolittle *et al.*, 1983; Robbins *et al.*, 1983), and in addition, PDGF purified from porcine platelets has the structure of a B chain homodimer (Stroobant and Waterfield, 1984).

It was recently found that the different dimeric forms of PDGF have different functional activities. Thus, PDGF-AA has a considerably lower mitogenic activity, chemotactic activity and ability to cause actin rearrangement, than has PDGF-AB (Nistér *et al.*, 1988); it was suggested that this is due to different reactivities with two distinct receptor classes. Indications of the existence of more than one PDGF receptor type also came from experiments demonstrating that the different dimers have different ability to down-regulate PDGF receptors (Hart *et al.*, 1988; Nistér *et al.*, 1988).

In this communication, we provide direct evidence for the presence of two distinct PDGF receptor types on human fibroblasts. The receptors are distinguished by their different ligand binding specificities, their sizes as estimated by affinity labeling, and by their different reactivities with a recently established monoclonal antibody against a PDGF receptor purified from porcine uterus (Rönnstrand *et al.*, 1988).

Results

Cross-competition for binding to human foreskin fibroblasts of different dimeric forms of PDGF

PDGF-AB purified from human platelets and recombinant PDGF A and B chain homodimers were ^{125}I-labeled and analysed with regard to their binding to human foreskin fibroblasts at 0°C. All three dimeric forms of PDGF showed a specific binding to the cells. As shown in Figure 1, [^{125}I]PDGF-AB was most efficiently competed for by PDGF-AB, with a 50% reduction in binding at 5 ng/ml, but PDGF-AA and PDGF-BB also competed with half-maximal effects at about 30 and 60 ng/ml respectively. Also, [^{125}I]-PDGF-AA was most efficiently competed for by PDGF-AB, with half-maximal effect at 5 ng/ml. In addition, PDGF-AA and PDGF-BB competed; half-maximal effects were achieved at 10 and 30 ng/ml respectively. In contrast, [^{125}I]PDGF-BB was only marginally competed for by PDGF-AA, whereas PDGF-AB and PDGF-BB competed with half-maximal effects at 40 and 60 ng/ml respectively.

These results indicate that at least two PDGF receptor classes exist on human fibroblasts. One receptor showed highest affinity for PDGF-AB, but bound also PDGF-AA and PDGF-BB. This receptor is tentatively called PDGF receptor type A. The other receptor bound PDGF-BB and PDGF-AB, but not PDGF-AA. This receptor is tentatively called PDGF receptor type B.

Binding of PDGF-AB and PDGF-BB to human fibroblasts after down-regulation with PDGF-AA

The result of the experiment described in Figure 1 indicates that PDGF-AB and PDGF-BB bind both to type A and type B receptors on human fibroblasts. In order to characterize

the binding of these ligands to the B type receptor only, the A type receptor was down-regulated by incubation of the fibroblasts with PDGF-AA for 1 h at 37°C. A wash at pH 3.75, which would dissociate bound PDGF-AA from the receptor, did not expose any A type receptor on such cells, indicating that the receptor was down-regulated rather than remaining at the cell surface but blocked by an excess of ligand (not shown). Dose–response analysis revealed that 50 ng/ml of PDGF-AA caused maximal down-regulation (not shown); this concentration was therefore used. After preincubation, the cells were cooled to 0°C and the binding of [^{125}I]PDGF-AB and [^{125}I]PDGF-BB determined. As shown in Figure 2A, preincubation with PDGF-AA resulted in a reduction of [^{125}I]PDGF-AB binding to about 25% of the control value. This effect was interpreted as resulting from a down-regulation of type A receptors. The binding of [^{125}I]PDGF-AB to the remaining B type receptor was of low affinity; 100 ng/ml of PDGF-AB reduced the binding of [^{125}I]PDGF-AB from 25 to 17%, i.e. by only 30% (Figure 2A). In contrast, as was expected from the experiment described in Figure 1, preincubation with PDGF-AA had very little effect on the binding of [^{125}I]PDGF-BB (Figure 2B). Thus, the B type receptor binds primarily PDGF-BB, and PDGF-AB with lower affinity.

Dissociation of [^{125}I]PDGF-AB and [^{125}I]PDGF-BB from human fibroblasts

Prebound [^{125}I]PDGF-AB has been found to dissociate very slowly from the surface of human fibroblasts upon incubation at 0°C (Heldin *et al.*, 1982). To investigate which one of the two PDGF receptor types is involved in the stabilization of binding, the dissociation of [^{125}I]PDGF-AB from human fibroblasts was compared with that from fibroblasts preincubated with PDGF-AA at 37°C to down-regulate the A type receptor. PDGF-AA pretreated cells and control cells were incubated with [^{125}I]PDGF-AB at 0°C, washed and further incubated at 0°C with increasing concentrations of unlabeled PDGF-AB; after 60 min of incubation the amount of [^{125}I]PDGF-AB released into the medium was determined. Only a small fraction (~20%) of bound [^{125}I]-PDGF-AB was displaced and there was essentially no effect of addition of unlabeled PDGF-AB to the medium, or by preincubation with PDGF-AA (Figure 3A). A similar experiment with [^{125}I]PDGF-BB gave essentially the same result; the displacement of [^{125}I]PDGF-BB was not affected by the addition of unlabeled PDGF-BB, nor by preincubation with PDGF-AA (Figure 3B). This indicates that the binding of

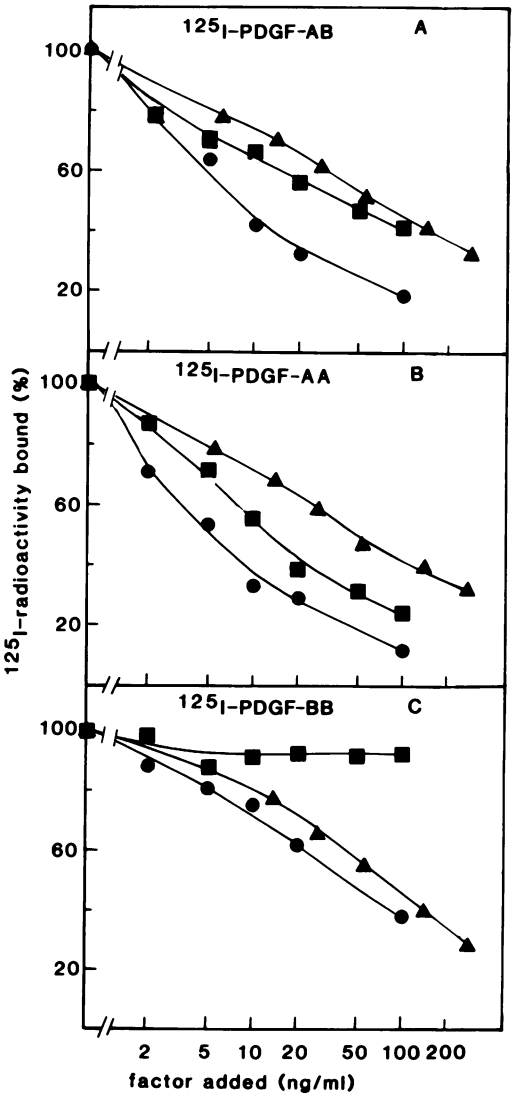

Fig. 1. Cross-competition for binding to human fibroblasts of different dimeric forms of PDGF. The binding of ^{125}I-labeled PDGF-AB (**A**), PDGF-AA (**B**) and PDGF-BB (**C**) to human fibroblasts at 0°C was determined in the presence of increasing concentrations of PDGF-AB (●—●), PDGF-AA (■—■) and PDGF-BB (▲—▲). The binding in the absence of unlabeled ligands (2400 c.p.m. [^{125}I]PDGF-AB; 2900 c.p.m. [^{125}I]PDGF-AA; 3200 c.p.m. [^{125}I]PDGF-BB) were set at 100%.

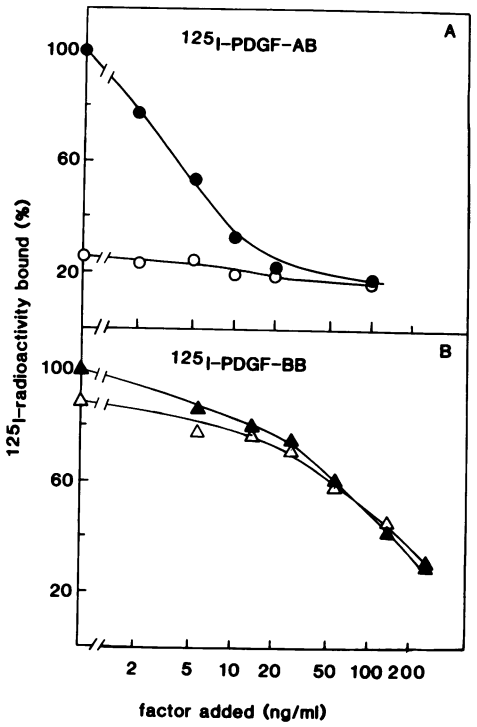

Fig. 2. Binding of PDGF-AB and PDGF-BB to human fibroblasts after down-regulation with PDGF-AA. The binding of [^{125}I]PDGF-AB (**A**) and [^{125}I]PDGF-BB (**B**) to human fibroblasts at 0°C was determined in the presence of increasing concentrations of the corresponding unlabeled ligands on cells incubated in the absence (closed symbols) or presence (open symbols) of 50 ng/ml PDGF-AA for 1 h at 37°C. 100% binding refers to 3000 c.p.m. [^{125}I]PDGF-AB and 4000 c.p.m. [^{125}I]PDGF-BB bound.

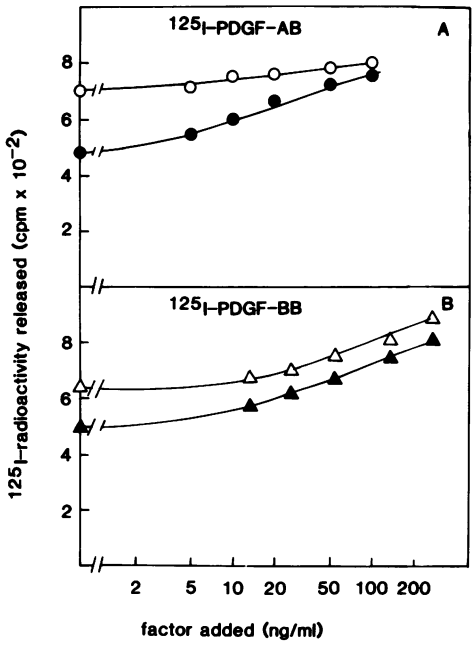

Fig. 3. Dissociation of [^{125}I]PDGF-AB and [^{125}I]PDGF-BB from human fibroblasts. [^{125}I]PDGF-AB (**A**) and [^{125}I]PDGF-BB (**B**) were bound for 2 h at 0°C to human fibroblasts that had been preincubated in the absence (closed symbols) or presence (open symbols) of 50 ng/ml PDGF-AA for 1 h at 37°C. After washing, incubation was continued at 0°C in the presence of increasing concentrations of the corresponding ligands; after 1 h the radioactivity released into the medium was determined. Total binding of [^{125}I]PDGF-AB was 2000 and 3500 c.p.m. on cells that were not preincubated with PDGF-AA respectively; the corresponding binding of [^{125}I]PDGF-BB was 1800 and 3000 c.p.m. respectively.

[^{125}I]PDGF-AB and [^{125}I]PDGF-BB to the B type receptor undergoes stabilization after binding, which then prevents their dissociation from the receptor.

Affinity cross-linking of different ^{125}I-labeled dimeric forms of PDGF

Affinity cross-linking using the homobifunctional cross-linker disuccinimidyl suberate (DSS) was used to characterize the sizes of the A and B types of PDGF receptors. The different ^{125}I-labeled dimeric forms of PDGF were bound to fibroblasts at 0°C; after washing, DSS was added to the cell cultures in order to cause covalent binding of the ligands to their receptors. Analysis by SDS–gel electrophoresis and autoradiography revealed that [^{125}I]PDGF-AA was cross-linked in a major complex of 140 kd and a minor one of 175 kd, [^{125}I]PDGF-BB was cross-linked to form complexes of 175–190 kd, whereas [^{125}I]PDGF-AB formed complexes of both 175–190 kd and 140 kd (Figure 4). In all cases, addition of an excess of the corresponding unlabeled ligand during incubation caused a decrease in the amount of radioactivity associated with high mol. wt components, indicating specific interactions between ligands and receptors.

The three ligands were also bound and cross-linked to cells that had been preincubated at 37°C with PDGF-AA, to remove the A type receptor. On these cells, [^{125}I]PDGF-AA was not cross-linked to any high mol. wt complex, whereas the cross-linking of [^{125}I]PDGF-BB to form the 175–190 kd complex remained (Figure 4). This is consistent with the conclusion that the 140 kd component contains the A type receptor and the 175–190 kd component(s) the B type receptor.

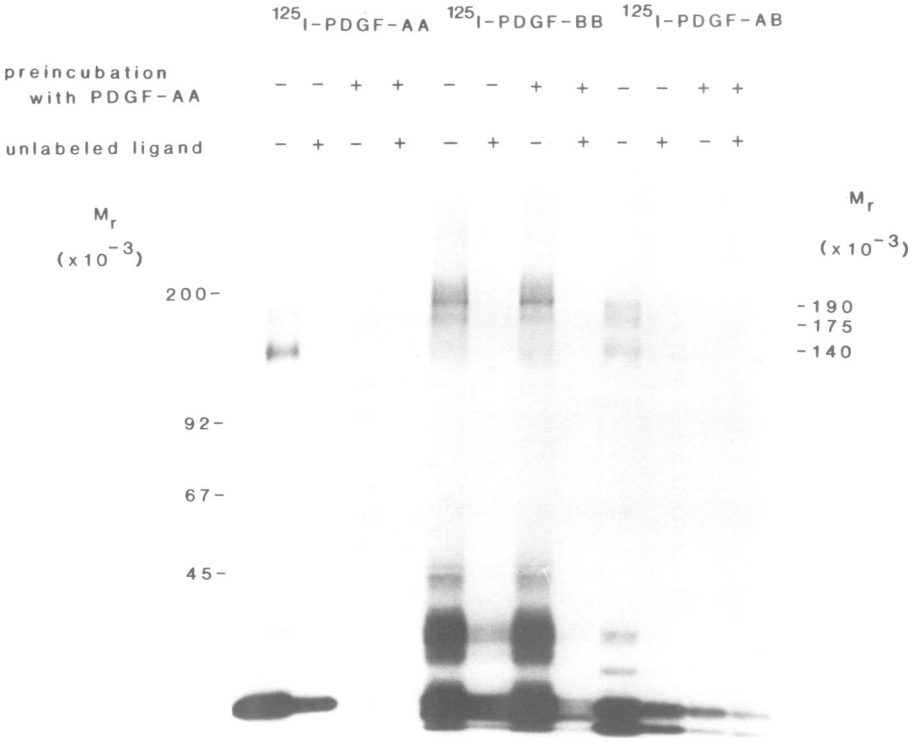

Fig. 4. Affinity cross-linking of different ^{125}I-labeled dimeric forms of PDGF to human fibroblasts. [^{125}I]PDGF-AB, [^{125}I]PDGF-AA and [^{125}I]PDGF-BB were incubated for 2 h at 0°C with cells that had been preincubated for 1 h at 37°C with or without 50 ng/ml PDGF-AA. After washing, the ^{125}I-labeled ligands were cross-linked with 0.05 mM DSS for 30 min at 0°C; samples were analysed by SDS–gel electrophoresis and autoradiography. As a control of specificity the bindings were performed in the absence or presence of 50 ng/ml of the corresponding unlabeled ligands.

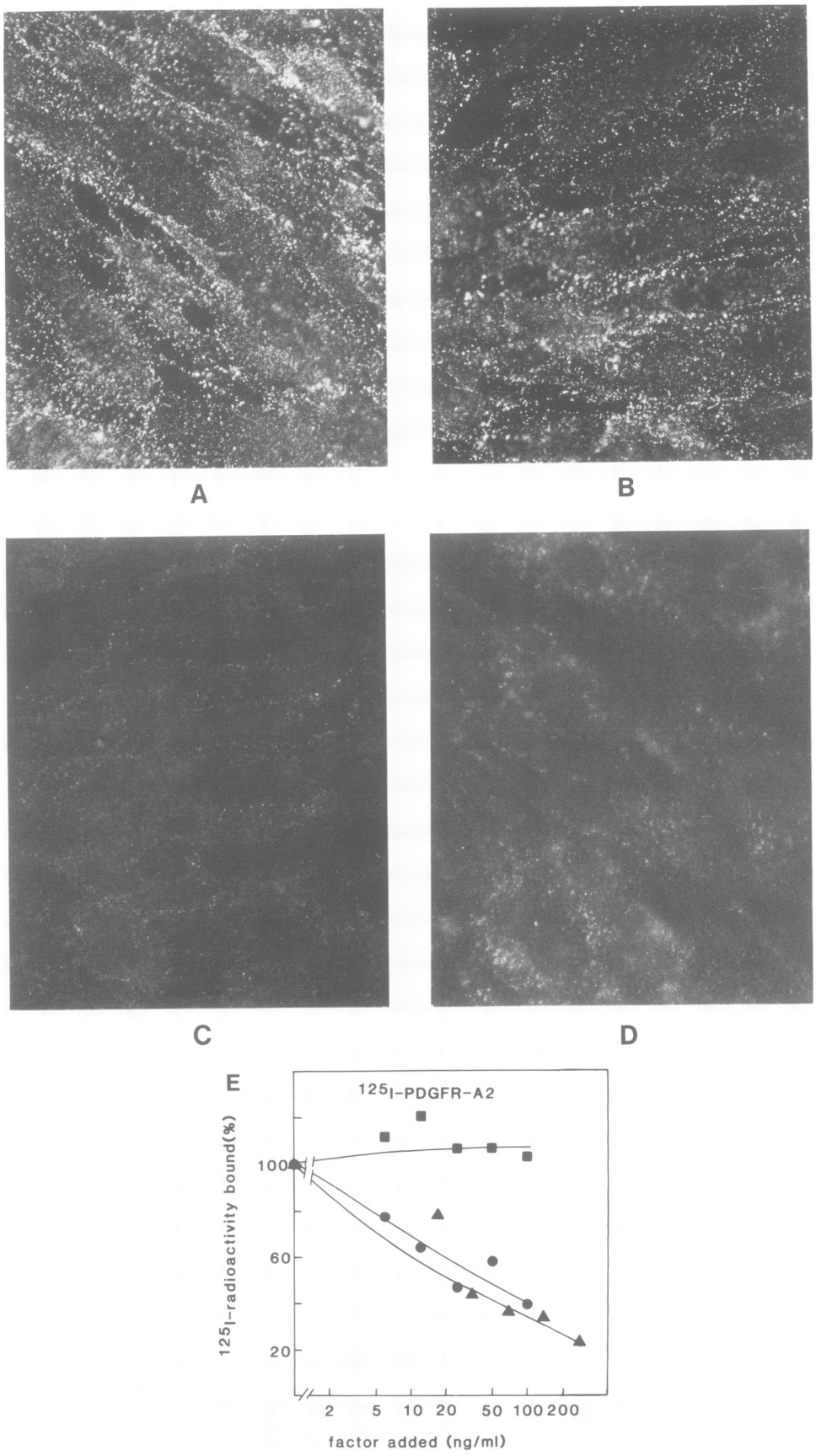

Fig. 5. Binding of monoclonal antibody PDGFR-B2 to human fibroblasts pretreated with various dimeric forms of PDGF. A PDGF receptor monoclonal antibody, PDGFR-B2, was used for immunofluorescence staining of human fibroblasts that had been preincubated at 37°C for 30 min with control buffer (**A**), or 100 ng/ml of PDGF-AA (**B**), PDGF-AB (**C**) or PDGF-BB (**D**). The binding of ^{125}I-labeled PDGFR-B2 at 0°C to cells that had been preincubated with increasing concentrations of PDGF-AB (●—●), PDGF-AA (■—■) or PDGF-BB (▲—▲) was also determined (**E**). 100% corresponds to 810 c.p.m. bound.

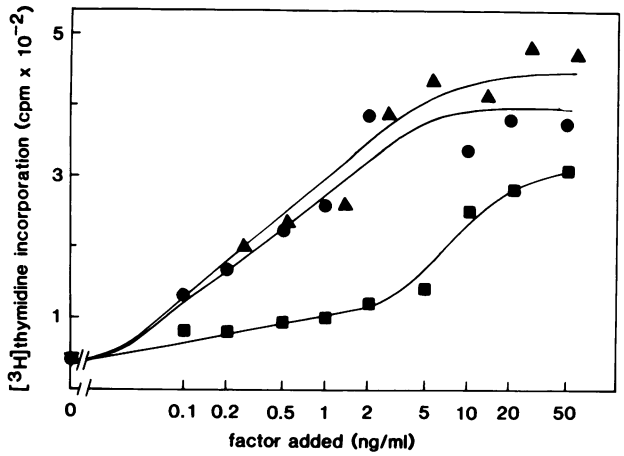

Fig. 6. Mitogenic activity of the different dimeric forms of PDGF. Increasing concentrations of PDGF-AB (●—●), PDGF-AA (■—■) or PDGF-BB (▲—▲) were assayed for the ability to stimulate incorporation of [^{3}H]TdR into human foreskin fibroblasts.

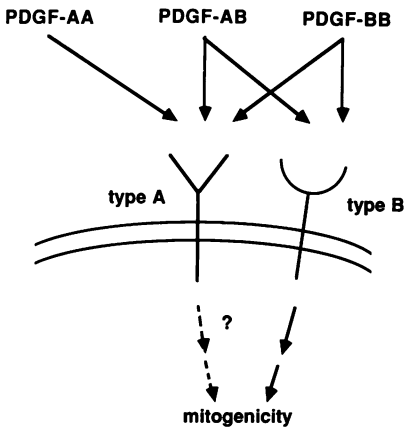

Fig. 7. Schematic illustration of the two different PDGF receptor types on human foreskin fibroblasts.

Binding of monoclonal antibody PDGFR-B2 to human fibroblasts pretreated with various dimeric forms of PDGF

Two monoclonal antibodies against the PDGF receptor purified from porcine uterus were recently established; both recognize the external domain of a human PDGF receptor (Rönnstrand *et al.*, 1988). One of these antibodies, PDGFR-B2, was investigated for its reactivity with A and B types of PDGF receptors. Human fibroblasts were preincubated for 60 min at 37°C with 100 ng/ml of PDGF-AA, PDGF-AB or PDGF-BB to down-regulate the corresponding receptors. As shown in Figure 5A, PDGFR-B2 gave a positive immunofluorescence staining on control cells. Preincubation with PDGF-AB (Figure 5C) or PDGF-BB (Figure 5D) quenched the immunofluorescence staining completely, but preincubation with PDGF-AA (Figure 5B) was essentially without effect. In order to quantify the binding of PDGFR-B2 antibody, the binding of ^{125}I-labeled PDGFR-B2 was determined on human fibroblasts preincubated at 37°C with different concentrations of the various dimeric forms of PDGF. PDGF-BB and PDGF-AB both caused half-maximal down-regulation at 30 ng/ml (Figure 5E). These results indicate that the PDGFR-B2 antibody recognizes the B type but not the A type of PDGF receptors, and that the previously purified PDGF receptor from porcine uterus (Rönnstrand *et al.*, 1987) is of B type.

Mitogenic activity of the different dimeric forms of PDGF

PDGF-AA, PDGF-AB and PDGF-BB were tested for their abilities to stimulate incorporation of [^{3}H]TdR into human fibroblasts. As shown in Figure 6, PDGF-AB and PDGF-BB were the most potent growth factors, half-maximal effects were achieved at about 1 ng/ml, whereas PDGF-AA was less potent, reaching the same degree of stimulation at 10 ng/ml. In addition, the maximal stimulation obtained with PDGF-AA was always lower than that of PDGF-BB or PDGF-AB.

Discussion

The present communication presents evidence that two distinct PDGF receptor classes exist that display differences in their binding of the three dimeric forms of PDGF. One receptor class, denoted type A, binds all dimeric forms of PDGF. The other receptor class, denoted type B, shows high affinity binding of PDGF-BB. It also binds PDGF-AB with lower affinity, but does not bind PDGF-AA. Figure 7 depicts schematically the two PDGF receptors and their ligand binding specificities.

A protein tyrosine kinase PDGF receptor (reviewed in Heldin and Rönnstrand, 1988) has been purified from 3T3 cells (Daniel *et al.*, 1985; Bishayee *et al.*, 1986) and porcine uterus (Rönnstrand *et al.*, 1987). cDNA for this receptor type (Yarden *et al.*, 1986; Claesson-Welsh *et al.*, 1988) has been cloned, and expressed in CHO cells; the transfected cells bound [^{125}I]PDGF-BB with high affinity, [^{125}I]PDGF-AB with lower affinity, but not [^{125}I]PDGF-AA (Claesson-Welsh *et al.*, 1988), indicating that the cloned PDGF receptor is of the B type. The monoclonal antibodies that have been established against purified receptor preparations (Hart *et al.*, 1987; Rönnstrand *et al.*, 1988) are also directed against the B type receptor (Hart *et al.*, 1988; this work).

The size of the B type receptor is known from the predicted amino acid sequence of its cDNA clone (Yarden *et al.*, 1986; Claesson-Welsh *et al.*, 1988), from the sizes in SDS/gel electrophoresis of components immunoprecipitated with specific antisera from metabolically labeled cells (Claesson-Welsh *et al.*, 1987; Hart *et al.*, 1987; Keatings and Williams, 1987), and of the purified receptor protein (Daniel *et al.*, 1985; Bishayee *et al.*, 1986; Rönnstrand *et al.*, 1987); the M_r of the polypeptide is 120 000 and the mature receptor, after post-translational modifications, has an apparent M_r in SDS/gel electrophoresis of 170 000−185 000. The determination of the size of the B type receptor by affinity labeling, 160−175 kd, after subtraction of the mol. wt of the ligand (Figure 4), conforms to these estimations. Previous estimates of the size of the PDGF receptor by affinity cross-linking using ^{125}I-labeled PDGF of unspecified dimeric forms yielded estimations of 163−185 kd (Glenn *et al.*, 1982; Heldin *et al.*, 1983; Williams *et al.*, 1984).

The major part of [^{125}I]PDGF-AA was cross-linked to form a complex of 140 kd, but a minor part also formed a complex of 175 kd (Figure 4). It is possible that the smaller molecule is formed from the larger by proteolysis; alternatively, the larger component reflects low affinity binding of [^{125}I]PDGF-AA to the B type receptor, or to another

component. Subtraction of the mol. wt of the ligand would yield a size for the type A receptor of 125 kd and of 160 kd for the larger molecule. Analysis by SDS–gel electrophoresis under non-reducing conditions revealed that the complex between [^{125}I]PDGF-AA and the receptor migrated slightly faster than under reducing conditions (125 versus 140 kd; not shown), indicating that the A type receptor, like the B type receptor, is a single chain protein which contains intra-chain disulphide bonds.

Certain growth factor receptor-like proto-oncogene products in this size range, with unknown ligands, have been described (reviewed in Westermark and Heldin, 1988). It remains to be seen whether any of them correspond to the A type PDGF receptor.

The affinity labeling experiment clearly indicated that the [^{125}I]PDGF-AB bound both to the A type (140 kd complex) and B type (175–190 kd complex) receptor. Preincubation of human fibroblasts with PDGF-AA, as expected, reduced the binding of the A type receptor. However, the binding to the B type receptor was also also reduced (Figure 4), suggesting that the presence of the A type receptor influenced the binding of [^{125}I]PDGF-AB to the B type receptor. The explanation for this finding is not known. PDGF induces dimerization of purified receptors after binding (unpublished observations). It is a possibility that the PDGF molecule might bind with higher affinity to one A and one B type receptor than to two receptors of the same type; removal of the A type receptor would then also decrease the binding of PDGF-AB to the B type receptor. Such a possibility is also supported by the fact that PDGF-AB was almost as potent as PDGF-BB in down-regulating the B type receptor on fibroblasts in spite of its lower affinity (Figure 2), as determined by the binding of the B type receptor antibody PDGFR-B2 (Figure 5).

In view of the fact that PDGF-AA, which seems to bind solely to the type A receptor, only has a limited mitogenic effect, it is likely that the B type receptor has a major role in the mediation of the mitogenic effect of PDGF. The low mitogenic effect of PDGF-AA might indicate that the A type receptor also mediates a mitogenic signal, albeit less efficiently. This possibility is also supported by the finding that PDGF-AB is as good a mitogen as PDGF-BB, in spite of the fact that PDGF-BB binds with a higher affinity to the B type receptor. It has not been excluded, however, that the mitogenic activity of PDGF-AA is mediated, directly or indirectly, via the B type receptor.

PDGF-AB and PDGF-BB, but not PDGF-AA, stimulate chemotaxis and actin reorganization leading to membrane ruffling (Nistér *et al.*, 1988; A.Siegbahn *et al.*, unpublished; K.Mellström *et al.*, unpublished). In fact, PDGF-AA inhibits PDGF-AB induced chemotaxis and, under certain conditions, membrane ruffling (Nistér *et al.*, 1988). This indicates that stimulation of chemotaxis and membrane ruffling are mediated by the B type receptor and that signals mediated by the interaction with the A type receptor, under certain conditions, may be inhibitory. The observation that PDGF-AA can transmodulate the EGF receptor (Nistér *et al.*, 1988), an effect which is mediated by activation of protein kinase C and phosphorylation of Thr-654 of the EGF receptor (Davis and Czech, 1985), suggests that the A receptor may be linked to the phosphatidylinositol cycle.

Expression of PDGF A chain mRNA and secretion of PDGF-AA have been found in many normal and transformed cell types. As PDGF-AA seems to mediate its effects via the A type receptor only, an important goal for future studies will be to characterize molecularly this receptor and identify the signals it transduces.

Materials and methods

PDGF

PDGF-AB was purified from human platelets as described (Heldin *et al.*, 1987). PDGF-AA and PDGF-BB were purified to apparent homogeneity from supernatants of yeast cells transfected with PDGF A chain and PDGF B chain constructs respectively (to be published). The concentrations of factors were determined by amino acid composition analysis. Some experiments were done with a partially purified batch of PDGF-BB.

PDGF-AA and PDGF-AB were ^{125}I-labeled with the chloramine T method (Hunter and Greenwood, 1982) to specific activities of 50 000 and 40 000 c.p.m./ng respectively. [^{125}I]PDGF-BB, labeled by the Bolton and Hunter method (1973) to a specific activity of 70 000 c.p.m./ng, was a kind gift of Amersham International.

Binding experiments

Binding experiments were performed on human foreskin fibroblasts (AG 1523, purchased from the Human Mutant Cell Repository, Camden, NJ), as described (Nistér *et al.*, 1984). Cells were grown in Linbro 12-well plates to confluence. Before binding, the cell cultures were rinsed once with 1 ml of binding buffer (PBS containing 1 mg/ml of bovine serum albumin, 0.01 mg/ml $CaCl_2.2H_2O$ and 0.01 mg/ml $MgSO_4.7H_2O$). Cell cultures were then incubated for 2 h at 0°C in 0.5 ml binding buffer containing 1–2 ng/ml of radiolabeled ligands and various concentrations of unlabeled ligands. Cultures were washed five times in ice-cold binding buffer; cells with associated ^{125}I-radioactivity were thereafter extracted by incubation for 30 min at room temperature in 0.5 ml Triton X-100, 20 mM Hepes, pH 7.4, 10% (v/v) glycerol, and determined in a gamma counter.

For down-regulation of the A type receptor, the fibroblast cultures were preincubated for 1 h at 37°C with 50 ng/ml of PDGF-AA. In some experiments, the cells were exposed for 1 min to binding buffer, supplemented with 20 mM acetic acid, pH 3.75, to dissociated receptor bound PDGF-AA at the cell surface. Cells were then washed in binding buffer at neutral pH.

Affinity cross-linking experiments

Human foreskin fibroblasts were grown to confluence on 6 cm Falcon dishes and incubated in the absence or presence of 50 ng/ml of PDGF-AA for 1 h at 37°C. After washing once in binding buffer, the cell cultures were incubated for 2 h at 0°C in 2 ml binding buffer containing 500 000 c.p.m. of ^{125}I-labeled PDGF-AA, PDGF-AB or PDGF-BB in the absence or presence of 50 ng/ml of the corresponding unlabeled ligand. After washing three times in ice-cold binding buffer and twice in PBS, cells were incubated for 20 min at 0°C in PBS containing 0.05 mM DSS. The cross-linking reaction was quenched by addition of 10 mM Tris base. After 10 min of incubation at 0°C, the cells were washed once in PBS containing 1 mM EDTA; the cells were then scraped off the plate with a rubber policeman in PBS containing 1 mM EDTA. Cells were pelleted by centrifugation for 5 min at 10 000 *g* and then solubilized for 30 min at 0°C in 1% Triton X-100, 20 mM Hepes, pH 7.4, 10% glycerol. After centrifugation for 5 min at 10 000 *g*, the supernatant was mixed with reducing SDS-sample buffer (Blobel and Dobberstein, 1975), heated at 95°C for 3 min, alkylated for 15 min at room temperature in 25 mM iodoacetamide, and analysed by SDS–gel electrophoresis.

SDS – gel electrophoresis

SDS–gel electrophoresis was performed as described (Blobel and Dobberstein, 1975) using gradient gels of 4–12% polyacrylamide.

Binding of monoclonal antibody

Human fibroblasts were grown to confluence on glass coverslips. After incubation for 1 h at 37°C in the absence or presence of 100 ng/ml of PDGF-AA, PDGF-AB or PDGF-BB, cells were washed once in serum-free Eagle's medium and incubated for 30 min at room temperature in serum-free Eagle's medium supplemented with 50 μg/ml of monoclonal antibody PDGFR-B2. Cells were then washed three times in serum-free Eagle's medium and incubated in a 1/40 dilution of FITC-labeled goat anti-mouse Ig (Beckton Dickinson) in serum-free Eagle's medium for 1 h at room temperature. After washing three times in PBS, cells were fixed in 2% *p*-formaldehyde in PBS. Cell cultures were inspected in a Nikon microscope equipped for epifluor-

escence and photographed using Kodak T-max film. Identical exposures were used to facilitate comparisons.

The binding of PDGFR-B2 to human fibroblasts was also determined by experiments utilizing ^{125}I-labeled PDGFR-B2 (labeled to a specific activity of 2200 c.p.m./ng by use of Iodobeads (Markwell, 1982). Confluent cell cultures in 12-well Linbro plates were preincubated in different concentrations of PDGF-AA, PDGF-AB or PDGF-BB for 1 h at 37°C, then washed once in 20 mM acetic acid, 0.15 M NaCl, 2.5 mg/ml bovine serum albumin, pH 3.75, to remove surface bound ligands, followed by one wash in binding buffer and then incubated for 2 h at 0°C in 0.5 ml binding buffer containing 1.1×10^6 c.p.m. [^{125}I]PDGFR-B2. Cells were then washed and cell associated radioactivity determined as described above. Non-specific binding was determined by inclusion of a 100-fold excess of unlabeled PDGFR-B2, and subtracted from the values of total binding.

Mitogenic assay

The mitogenic activity of PDGF-AB, PDGF-AA and PDGF-BB was determined as the ability to stimulate incorporation of [^{3}H]TdR into human fibroblasts (Betsholtz and Westermark, 1984).

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