Full Activation of the Platelet-derived Growth Factor β-Receptor Kinase Involves Multiple Events*

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Activation of receptor tyrosine kinases is thought to involve ligand-induced dimerization, which promotes receptor transphosphorylation and thereby increases the receptor's phosphotransferase activity. We used two platelet-derived growth factor β-receptor (β-PDGFR) mutants to identify events that are required for full engagement (autophosphorylation and activation of the kinase) of the β-PDGFR kinase. The F79/81 receptor (Tyr to Phe substitution at 579 and 581 in the juxtamembrane domain of the receptor) was capable of only very modest ligand-dependent autophosphorylation and also failed to associate with numerous SH2 domain-containing proteins. Furthermore, stimulation with platelet-derived growth factor (PDGF) did not increase the kinase activity of the F79/81 mutant toward exogenous substrates. However, the F79/81 receptor had basal kinase activity and could be artificially stimulated by incubation with ATP. Because the low kinase activity of the F857 mutant (Tyr to Phe substitution at 857 in the putative activation loop) could not be increased by incubation with ATP, failure to tyrosylate Tyr-857 may be the reason why the F79/81 mutant has low kinase activity. Surprisingly, the F857 mutant underwent efficient PDGF-dependent autophosphorylation. Thus the PDGF-dependent increase in the kinase activity of the receptor is not required for autophosphorylation. We conclude that full activation of the β-PDGFR kinase requires at least two, apparently distinct events.

Receptor tyrosine kinases are a large family of transmembrane-spanning proteins that transmit signals from the extracellular environment by means of activating their intrinsic kinase activity. The ligand for the platelet-derived growth factor β-receptor (β-PDGFR) exists as a dimer, and on binding to the receptor it induces dimerization (1). Much work has indicated that dimerization is both necessary and sufficient to activate the kinase activity of the receptor, leading to autophosphorylation of the receptor and the subsequent binding and phosphorylation of downstream signaling proteins (reviewed in Refs. 2–5), but the precise steps involved in the mechanism of receptor activation remain unknown. Receptor activation is the first step in the increasingly complex signaling cascades induced by receptor tyrosine kinases; therefore a complete understanding of the activation process is important.

Many tyrosines in the intracellular portion of the β-PDGFR have been shown to be phosphorylated in response to ligand binding to the extracellular portion of the receptor. Phosphorylation of tyrosine 857 in the putative kinase activation loop has been shown to be required for maximal receptor kinase activity (6, 7). Many other phosphorysines act as docking sites for SH2 domain-containing proteins such as the GTPase-activating protein of Ras (RasGAP) (8), phospholipase C γ-1 (PLCγ) (9–12), and the p85 subunit of PI 3-kinase (p85) (13–16). PDGF-dependent association of Src family members requires tyrosines 579 and 581 in the juxtamembrane region of the human β-PDGFR (17, 19). Mutation of either tyrosine 579 or 581 individually led to a decrease in Src association with the receptor (17), whereas mutation of both sites led to a receptor that failed to detectably associate Src (19) and has been reported to be devoid of ligand-stimulatable kinase activity (17).

We constructed and characterized a β-PDGFR mutant with tyrosines 579 and 581 mutated to phenylalanine (referred to as F79/81) and confirmed the finding that these sites are required for association of Src with the receptor (17–19). In addition we found that the β-PDGFR did not become efficiently tyrosine-phosphorylated in response to PDGF and as a result was unable to recruit wild type levels of SH2 domain-containing proteins. However, in contrast to previous findings (17) we found that the F79/81 receptor was not devoid of kinase activity, with the basal phosphorylation of the F79/81 receptor being comparable or slightly higher than that of the WT receptor. In addition we found that the F79/81 receptor kinase activity could be artificially activated by incubation with ATP. To investigate the defect in the ligand-induced activation of the F79/81 receptor we compared it to another β-PDGFR mutant where tyrosine 857 was replaced with phenylalanine. In contrast to the F79/81 receptor, the F857 receptor became highly tyrosine-phosphorylated following exposure to PDGF. However, despite being highly tyrosine-phosphorylated the F857 receptor was not activated as a kinase toward exogenous substrates. Comparison between the F79/81 and F857 receptors indicated that there are at least two distinct steps in receptor activation, autophosphorylation that requires tyrosines 579 and 581 in the juxtamembrane region and phosphorylation of tyrosine 857 that allows the receptor to phosphorylate exogenous substrates.

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1 The abbreviations used are: β-PDGFR, platelet-derived growth factor β-receptor; PDGF, platelet-derived growth factor; F79/81, tyrosines 579 and 581 mutated to phenylalanine; F857, tyrosine 857 mutated to phenylalanine. PLC, phospholipase C; PI, phosphatidylinositol; WT, wild type; BSA, bovine serum albumin; Pipes, 1,4-piperazineethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; GST, glutathine; one S-transferase; R634, kinase-inactive receptor where lysine at 634 is mutated to arginine.
Activation of the β-PDGF Kinase

MATERIALS AND METHODS

Cell Lines—HepG2 cells, a human hepatoma cell line that does not express endogenous β-PDGF receptors, were cultured and maintained as described previously (20).

Construction and Expression of β-PDGF Receptor Mutants—Substitutions of phenylalanine for tyrosine at positions 579 and 581 in the human β-PDGF receptor were performed by site-directed mutagenesis as described previously (19). The mutated receptor DNA was subcloned into the pLXSN2 vector and introduced into the ϕ2 and PA317 packaging cell lines, and the resulting virus was used to infect parental HepG2 cells (20). The infected cells were selected in the presence of 1 mg/ml (active concentration) G418, and the resulting mass populations were sorted by fluorescence-activated cell sorter using the human β-PDGF receptor-specific monoclonal antibody 7R7212 (Genzyme) to obtain cell lines expressing comparable levels of receptors, previously estimated to be 5 × 10^5 receptors/cell (20).

Immunoprecipitation and Western Blotting—HepG2 cells were grown to 80% confluence and then incubated for 16–24 h in Dulbecco’s modified Eagle’s medium containing 0.1% fetal bovine serum. Cells were then treated with 40 ng/ml PDGF-BB or with buffer alone at 37 °C for the indicated times. Cells were washed twice with HS (20 mM Hepes, pH 7.4, 150 mM NaCl) and then lysed in EB (10 mM Tris-HCl, pH 7.4, 5 mM EDTA, 50 mM NaCl, 50 mM NaF, 1% Triton X-100, 0.1% BSA, 20 μg/ml aprotinin, 2 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride) (22). Lysates were centrifuged for 20 min at 12,000 × g, and receptors were immunoprecipitated from the soluble fraction with the 30A anti-serum, which is specific for the β-PDGFR (24). Immune complexes were boiled for 10 min in formalin-fixed membranes of Staphylococcus aureus, spun through an EB sucrose gradient, and then washed twice with EB, PAN (10 mM Pipes, pH 7.0, 100 mM NaCl, 1% aprotinin) + 0.5% Nonidet P-40, and PAN (23) and resuspended in PAN before being used in kinase assays or analyzed by Western blotting.

To immunoprecipitate Src, resting or stimulated HepG2 cells were lysed in buffer containing 20 mM Tris-HCl, pH 7.4, 30 mM NaHPO4, 50 mM NaF, 40 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.1% BSA, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM Na3VO4, and 5 μg/ml aprotinin. Lysates were spun for 10 min at 12,000 × g, and Src family members were precipitated from the supernatant using Src-2 antisera (Santa Cruz). Immune complexes were collected on protein A-Sepharose and washed three times with lysis buffer and twice with buffer lacking BSA.

For Western analysis, proteins were resolved by electrophoresis on 7.5% SDS-PAGE and transferred to Immobilon. Membranes were incubated for 1 h in TBS (10 mM Tris base, pH 8.0, 150 mM NaCl, 0.2% Tween 20) and 2% BSA for probing with antiphosphotyrosine or TBS + 5% nonfat dry milk for all other antibodies. The following primary antibodies were used: β-PDGFR, 30A thyro-1,000; antiphosphotyrosine 4G10 (UBI); P20 (Transduction) 1:1, 1:10,000; PLCγ (mixture of monoclonals from UBI) 0.25 μg/ml, p85 subunit of PI 3-kinase (UBI) 1:1,000, RasGAP 69.3 (24) 1:1,000. Secondary antibodies were horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse (Amersham Pharma- cia Biotech) diluted 1:2,000, and all blots were visualized using ECL detection.

Kinase Assays—β-PDGFIR immunoprecipitates were subjected to a standard in vitro kinase assay (6) in the presence of 100 μM peptide (MAEFEEYVPIAKK) or 0.5 μg of GST-PLCγ and 10 μCi of γ-[32P]ATP in universal kinase buffer (UKB) (20 mM Pipes, pH 7.0, 10 mM MnCl2, 20 μg/ml aprotinin). To pretreat samples with unlabeled ATP, immunoprecipitates were incubated with UKB containing 10 μM ATP for 20 min at 30 °C and then washed once with PAN before being subjected to an in vitro kinase assay. Phosphorylated peptides were separated from γ-[32P]ATP on cellulose plates by thin layer electrophoresis at pH 3.5, and phosphorylated GST-PLCγ was separated by 7.5% SDS-PAGE. The level of phosphorylation of peptide or GST-PLCγ was quantitated using Molecular Dynamics PhosphorImager IQ software.

Preparation of Pervanadate—Equal volumes of 20 mM H2O2 and 2 mM MnCl2 were combined at room temperature for 10 min. The resulting solution was added to intact cells to a final concentration of 1 mM H2O2 and 0.1 mM MnCl2.

RESULTS AND DISCUSSION

Tyrosines 579 and 581 in the juxtamembrane region of the human β-PDGFIR were mutated to phenylalanine (19), and the resulting receptor (F79/81) was expressed in HepG2 cells. HepG2 cells express a very low level of PDGF-α-receptor and no detectable β-PDGFIRs (29). It had been previously reported that mutation of tyrosines 579 and 581 resulted in a kinase-inactive receptor (17) or a receptor with reduced kinase activity (19). Therefore, we first compared the ability of the F79/81 receptor to become phosphorylated in response to PDGF with the WT receptor and a receptor with a mutation at tyrosine 857 (F857) previously shown to be required for full kinase activity (6, 7). HepG2 cells expressing these receptors were exposed to 40 ng/ml PDGF-BB for 5 min and then lysed and β-PDGFR-immunoprecipitated. A portion of the immunoprecipitates representing approximately 1.5 × 10^6 cells was analyzed for phosphotyrosine content and receptor levels by Western blot (Fig. 1). Fig. 1A shows that the WT receptor had undetectable levels of phosphorylation in resting cells and became highly tyrosine-phosphorylated after exposure to PDGF. Surprisingly the F857 receptor also became highly tyrosine-phosphorylated in response to PDGF, although levels of phosphorylation were slightly reduced compared with WT. Note that part of the apparently lower F857 phosphorylation compared with WT following PDGF stimulation was because of there being less F857 that WT receptor (Fig. 1B). In contrast to the WT and F857 receptors, the F79/81 receptor was very poorly phosphorylated following addition of PDGF despite approximately equal amounts of receptor being present in the samples (Fig. 1B). Increasing the length of time that the cells were exposed to PDGF had no effect on the level of tyrosine phosphorylation of the F79/81 receptor (data not shown).

Phosphorylation of the β-PDGFIR is required for the association of many SH2 domain-containing proteins. Our finding that the F79/81 receptor was poorly phosphorylated suggested that the Src family members may not be the only SH2-containing proteins that cannot associate efficiently with the F79/81 receptor. Consequently, we tested the ability of the F79/81 receptor to associate with a number of proteins that have been shown to bind to the WT receptor following PDGF stimulation. Receptors were immunoprecipitated from resting or stimulated cells and subjected to Western blot analysis for the β-PDGFR, PLCγ, RasGAP, and the p85 subunit of PI 3-kinase (p85). As shown in Fig. 2A, the F79/81 receptor bound reduced amounts of PLCγ and p85 and undetectable levels of RasGAP compared with the WT receptor. The F857 receptor bound the proteins to levels similar as the WT receptor. The kinase dead receptor (R634) included as a control did not detectably bind any of the proteins that associate with the WT receptor.
When expressed in other cell types (PC12 (19) or Ba/F3 cells (25)) the F79/81 receptor did show some receptor autophosphorylation and kinase activity, similar to the data presented here for HepG2 cells. Because none of the cell lines used for these studies naturally express the β-PDGFR, it is possible that they do not accurately reflect the behavior of the F79/81 receptor in a natural setting. We have expressed a chimeric α/β FT27/74 receptor (26) in Ph cells, which are a 3T3-like cell line that does not express the α-PDGFR, as well as in HepG2 cells. This chimeric receptor mutant behaved comparably when expressed in these two cell lines. These findings indirectly indicate that the behavior of the F79/81 receptor is not dramatically altered by expressing it in the HepG2 cells. Ongoing studies, in which the mutant receptors will be expressed in mesenchymal cells devoid of either α- or β-PDGFRs, will better address these issues.

One explanation for the failure of PDGF to promote the phosphorylation of the F79/81 receptor is that the mutant receptors are unable to dimerize correctly. This does not appear to be the case because the ligand binding domain is within the first three IgG loops in the extracellular domain, and mutations within the cytoplasmic domain have not been shown to inhibit ligand binding or dimerization of the receptors. Furthermore, mutation of tyrosine 579 or 581 alone had no effect on ligand binding (17). Finally, we found that the F79/81 and WT receptors underwent a comparable extent of the PDGF-dependent dimerization (data not shown). These data indicate that replacing tyrosines 579 and 581 with phenylalanine did not prevent binding of PDGF to the extracellular domain of the receptor or receptor dimerization.

A second possibility is that the phosphorylation of the juxtamembrane sites makes a very large contribution to the overall phosphorylation of the receptor. However, it has been shown that in vivo the most highly phosphorylated sites in the receptor are at tyrosines 857 and 751 (16). In addition, the phosphorylation of sites 579 and 581 was not detectable by standard phosphopeptide mapping techniques suggesting that they are phosphorylated to low stoichiometry in vivo (17). Mutation of tyrosine 751 alone or in combination with up to four other tyrosines involved in binding-associated proteins did not prevent the receptor from undergoing extensive tyrosine phosphorylation in vivo (27). Therefore it is unlikely that the decrease in phosphorylation of the F79/81 receptor is because of the lack of two phosphorylation sites.

Because the activation of receptor tyrosine kinases has been inextricably linked with receptor phosphorylation, we next tested the kinase activity of the β-PDGFR mutants. WT, F857, and F79/81 receptors were immunoprecipitated from resting or PDGF-BB-stimulated cells, and immunoprecipitates representing approximately 3 × 10⁶ cells were subjected to an in vitro kinase assay in the presence of a peptide substrate with a tyrosine residue in a sequence shown to be the optimal phosphorylation site for the β-PDGFR (28). Peptides were separated from the γ-[³²P]ATP on cellulose plates by thin layer electrophoresis, and the degree of phosphorylation of the peptides was quantitated using a PhosphorImager. Fig. 3A shows that the WT receptor immunoprecipitated from PDGF-treated cells phosphorylates the peptide approximately four times better than receptors from resting cells. However, PDGF treatment of cells expressing the F79/81 or F857 receptors did not detectably increase the kinase activity of either receptor toward the peptide substrate. We altered the time of the assay incubation (Fig. 3C) and the substrate concentration (Fig. 3D), but neither of these variables improved the activity of the F79/81 or F857

*1* K. DeMali and A. Kazlauskas, unpublished observations.
receptors. In addition, increasing the time of exposure of the cells to PDGF prior to precipitation of the receptors did not result in activation of either the F79/81 or the F857 receptors (data not shown). We also tested the activity of the receptor mutants against a GST-PLCγ fusion protein substrate (29) and found WT receptor phosphorylated this substrate approximately six times better following PDGF stimulation. Addition of PDGF to cells expressing the F79/81 and F857 receptors had very little effect on the ability of either receptor to phosphorylate GST-PLCγ (Fig. 3).

Whereas the kinase activity of the F79/81 and F857 receptors was not increased in response to PDGF, these receptors did show basal tyrosine phosphorylation that was greater than that of the WT receptor (Fig. 1) and basal kinase activity that was comparable with the WT (data not shown). Because the receptors were not catalytically dead we tested whether they could be artificially activated. WT, F79/81, and F857 receptors were immunoprecipitated from resting cells and incubated with 10 μM ATP prior to being assayed for activity against the peptide substrate. Fig. 4 shows that incubation of F79/81 receptor with ATP caused the receptor to become activated to the same extent as the WT receptor. In contrast, the F857 receptor was poorly activated by this treatment. These data indicate that the F79/81 receptor is capable of being artificially activated. Furthermore, the failure of the F857 receptor to be activated by incubation with ATP suggests that the F79/81 receptor kinase is not activated in response to PDGF in vivo because it does not become phosphorylated at tyrosine 857.

A method to increase the phosphotyrosine content of proteins in vivo is to treat cells with the nonspecific tyrosine phosphatase inhibitor sodium pervanadate (30). We reasoned that this could be a way to phosphorylate and to potentially activate the F79/81 and F857 receptors in vivo. Cells were treated with sodium pervanadate for 2 min before being lysed, and then the b-PDGFRs were immunoprecipitated, and a portion of the immunoprecipitate representing 1.5 × 10^6 cells was analyzed for phosphotyrosine content by Western blotting and the blots stripped and reprobed for b-PDGFR. Fig. 5 shows that the WT, F79/81, and F857 receptors all became highly phosphorylated in sodium pervanadate-treated cells (Fig. 5A). The apparently lower extent of phosphorylation of the F857 receptor with all treatments (Fig. 5A) was because of a reduced amount of receptor in the immunoprecipitates (Fig. 5B). Thus pervanadate treatment of cells was able to stimulate robust tyrosine phosphorylation of all the receptors. We next tested the receptors immunoprecipitated from sodium pervanadate-treated cells for

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**Fig. 3. Kinase activity of receptors.** Cells were starved, stimulated, and immunoprecipitated as described in the legend to Fig. 1. A, immunoprecipitates representing 3 × 10^5 cells were assayed against 100 μM peptide (MAEEVEVFIEAKKK) with 10 μCi of γ-[32P]ATP for 1 h at 30 °C. 20% of the reaction mixture was spotted onto cellulose thin layer plates and electrophoresed at pH 3.5 for 30 min. Phosphorylated peptides were quantitated using Molecular Dynamics PhosphorImager IQ software. Data presented are the mean ± S.D. from at least 12 independent experiments for each receptor type. B, immunoprecipitates representing 3 × 10^5 cells were assayed against 0.5 μg of GST-PLCγ with 10 μCi of γ-[32P]ATP for 10 min at 30 °C. Reaction products were separated on 7.5% SDS-PAGE, and the amount of phosphorylation of substrate was quantitated using Molecular Dynamics PhosphorImager IQ software. Data presented are the mean ± S.D. of four independent experiments. C, immunoprecipitates representing 3 × 10^5 cells were assayed against 100 μM peptide at 30 °C for the times indicated along the x axis and then treated as described in A. Squares, WT receptor; diamonds, F79/81 receptor; circles, F857 receptor. Data presented are the mean ± S.D. of three independent experiments. D, immunoprecipitates representing 3 × 10^5 cells were assayed against increasing concentrations of peptide indicated on the x axis for 1 h at 30 °C before being treated as described in A. Squares, WT receptor; diamonds, F79/81 receptor; circles, F857 receptor. Data presented are the mean ± S.D. from three independent experiments.
kinase activity toward the peptide substrate. Immunoprecipitates representing 3 × 10⁵ cells were assayed for activity against the peptide substrate. Fig. 5C shows that treatment of the cells with sodium pervanadate failed to increase the kinase activity of any of the receptors toward the peptide substrate. These data show that sodium pervanadate treatment of cells does not increase the kinase activity of the receptors toward exogenous substrates, despite extensive tyrosine phosphorylation of the receptors. Given that the phosphorylation of tyrosine 857 appears to be required for activating the receptor’s kinase activity toward exogenous substrates it is possible that this tyrosine is inaccessible for phosphorylation when receptors are monomeric.

If receptor dimerization was a prerequisite for phosphorylation of Tyr-857, then treating cells with pervanadate and PDGF should activate the F79/81 receptor. To test this possibility, cells were treated for 2 min with sodium pervanadate, and then the medium was removed and replaced with fresh medium containing PDGF for 5 min before cells were lysed and receptors immunoprecipitated. Fig. 5A shows that this treatment led to highly phosphorylated receptors, in the same way as sodium pervanadate treatment alone. This treatment also increased the kinase activity of the WT receptor to the same extent as PDGF alone (Fig. 5C), indicating that the phosphorylation of the receptor because of sodium pervanadate treatment does not prevent or enhance activation of the WT receptor kinase following ligand binding. However, pretreatment with sodium pervanadate followed by the addition of PDGF did not give any increase in the kinase activity of the F79/81 or F857 receptors. Fig. 5C also shows that the kinase-inactive form of the β-PDGFR (R634) became highly tyrosine-phosphorylated following sodium pervanadate treatment. This indicates that a kinase other than the receptor itself is responsible for the phosphorylation seen following sodium pervanadate treatment. In summary, the lack of activation of the F79/81 receptor following pervanadate and PDGF treatment could be because the sites that need to be phosphorylated to achieve an activated kinase are not phosphorylated in the presence of sodium pervanadate.

Because the levels of receptor phosphorylation and kinase activity are so profoundly affected by mutation of tyrosines involved in binding Src, it is possible that the defect in the F79/81 is because of a lack of Src association with the β-PDGFR. Previous studies have shown that Src will phosphorylate and activate the receptors for insulin and insulin-like growth factor 1 in vitro (31–33), and Src has been shown to phosphorylate tyrosine 934 of the β-PDGFR both in vitro and in vivo (34). In addition, receptor tyrosine kinases appear to be activated when c-Src is overexpressed or when activated forms of Src are expressed (35–37). However, under a variety of in vitro conditions we could not show β-PDGFR receptor tyrosine

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**Fig. 4. Activation of receptors with ATP.** Cells were starved and then left resting or stimulated with PDGF, and receptors were immunoprecipitated as described in the legend to Fig. 1. Immunoprecipitates from unstimulated cells were incubated with 10 μM ATP for 20 min at 30 °C and then washed once with PAN to remove the ATP. Immunoprecipitates representing 3 × 10⁵ cells were then tested for their ability to phosphorylate the peptide substrate analyzed as described in Fig. 3A. Open bars represent kinase activity following PDGF stimulation, and shaded bars represent kinase activity following incubation with ATP. Data presented are the mean ± S.D. from three independent experiments for WT and F79/81 and from four independent experiments for F857.

**Fig. 5. Effects of treating cells with pervanadate.** Cells were grown to 80% confluence and starved in 0.1% serum for 16–20 h before being treated with 40 ng/ml PDGF BB for 5 min, 0.1 mM pervanadate for 2 min (PV), or 0.1 mM pervanadate for 2 min; then the medium was removed and replaced with fresh medium containing 40 ng/ml PDGF BB for 5 min. Cells were lysed and receptors precipitated with 30A. Immunoprecipitates representing 1.5 × 10⁶ cells were subjected to Western blot analysis for phosphotyrosine levels (A), and then the blots were stripped and reprobed with 30A to determine the amount of receptor in each sample. (B, C), immunoprecipitates representing 3 × 10⁵ cells were assayed against peptide as described in the legend to Fig. 3A. Open bars represent kinase activity following PDGF treatment, lightly shaded bars represent kinase activity following addition of sodium pervanadate, and darkly shaded bars represent kinase activity following sodium pervanadate pretreatment followed by addition of PDGF. Similar Western blots were seen in three independent experiments, and the data in C are the mean ± S.D. from four independent experiments.

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**Table 1:**

|                | WT   | F79/81 | F857 | R634 |
|----------------|------|--------|------|------|
| PDGF:          | -    | +      | +    | +    |
| PV:            | -    | +      | +    | +    |
| Fold increase above basal | 10  | 7.5    | 5    | 2.5  |

**Note:**

|                | WT   | F79/81 | F857 | R634 |
|----------------|------|--------|------|------|
| PDGF:          | -    | -      | -    | -    |
| PV:            | -    | +      | +    | +    |
| Fold increase above basal | 10  | 7.5    | 5    | 2.5  |
phosphorylation by Src. Although this does not rule out a role for Src acting through intermediates or the possibility that Src phosphorylates the receptor in vivo, our data suggest that the role of tyrosines 579 and 581 in PDGF receptor activation is not a direct effect of receptor phosphorylation by Src.

Several groups have shown that phosphorylation at tyrosine 857 is required for full activation of the β-PDGFR receptor (6, 7). Based on the crystal structure of the receptors for insulin and fibroblast growth factor, kinase activity is suppressed by an “activation loop,” which sterically blocks the catalytic site (38, 39). Phosphorylation of one or more tyrosine residues in the activation loop removes the steric hindrance and allows substrates access to the catalytic site. More recent crystals of Src family members have suggested that the activation of the kinase involves movement of α-helix C, which leads to conformational changes that lead to increased kinase activity (40, 41). The phosphorylation of the activation loop may also be involved in the movement of α-helix C. The crystal structure of the β-PDGFR is yet to be solved, but based on sequence comparisons tyrosine 857 is located in a region homologous to the activation loop seen in other receptors, and a role for this site in receptor activation can be postulated. Consistent with tyrosine 857 being involved in repression of β-PDGFR kinase activity, when the site is mutated to phenylalanine (F857), the basal kinase activity of the F79/81 and F857 receptor mutants in activated toward exogenous substrates in response to PDGF. In addition to the defect in binding-SH2 domain-containing proteins, the F79/81 receptor fails to become efficiently phosphorylated in response to PDGF but that it is also deficient in associating with a number of other SH2 domain-containing proteins. The basis for the defect in association with SH2 domain-containing proteins appears to be that the F79/81 receptor fails to become efficiently phosphorylated in response to the binding of PDGF. In addition to the defect in binding-associated proteins, the F79/81 receptor kinase activity was not activated toward exogenous substrates in response to PDGF stimulation. Comparison of the tyrosine phosphorylation and kinase activity of the F79/81 and F857 receptor mutants in response to ligand stimulation leads us to propose a two-step model for the full activation of the PDGF receptor. This model proposes that the two juxtamembrane tyrosines at 579 and 581 are required for receptor autophosphorylation, which is a prerequisite for the later phosphorylation of tyrosine 857, which is necessary for the activation of the receptor kinase activity.

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