**INTRODUCTION**

PDGF is a homo- or hetero-dimeric protein composed of A- and B-polypeptide chains which binds to two structurally related tyrosine kinase receptors, designated α- and β-receptors (Claesson-Welsh et al., 1989; Matsui et al., 1989; Yarden et al., 1986). The PDGF α-receptor is able to bind both the A- and B-chains of PDGF, whereas the β-receptor binds only the B-chain (Heldin et al., 1988). The receptors are expressed on cells of mesenchymal origin, such as fibroblasts, glia cells and capillary endothelial cells, and on epithelial and neuronal cells (Heldin and Westermark, 1996).

The PDGF ligand-receptor pairs have been implicated in embryonal development and wound healing as well as several pathological conditions for example fibrosis, atherosclerosis, glomerulonephritis and malignancies (Heldin and Westermark, 1996; Raines et al., 1990). Mice carrying null mutations of either PDGF ligands or receptors have helped clarify the functional roles of these ligand-receptor pairs. Mice deficient for PDGF-B show renal, cardiovascular, and hematological abnormalities (Levéen et al., 1994). The phenotype of the PDGF β-receptor deficient mice is very similar (Soriano, 1994). On the other hand PDGF-A deficient mice show severe defects in alveologenesis (Boström et al., 1996). Deletion of the PDGF α-receptor gene is, however, embryonically lethal in mice, giving rise to skeletal defects and increased apoptosis along the migration pathway of cranial neural crest cells (Soriano, 1997).

Upon PDGF binding receptors dimerize, inducing receptor autophosphorylation and phosphorylation of intracellular substrates (Ullrich and Schlessinger, 1990). An important role for receptor autophosphorylation is to present binding sites for signal transduction molecules containing Src homology 2 (SH2) domains (Pawson, 1995). SH2 domains consist of approximately 100 amino acids which bind phosphorylated tyrosine residues within the context of a stretch of three to six amino acid residues specific for each SH2 domain, contained in activated receptors and cytoplasmic phosphoproteins. Several of the autophosphorylation sites in the PDGF receptors have been shown to interact in a specific manner with certain SH2 domain-containing proteins (Claesson-Welsh, 1994). Among the nine autophosphorylation sites identified in the PDGF α-receptor, Tyr-572 and Tyr-574, were identified. A Y572/574F mutant PDGF α-receptor was generated and stably expressed in porcine aortic endothelial cells. In contrast to the wild-type receptor, the mutant receptor was unable to associate with or activate Src family tyrosine kinases. Tyrosine phosphorylated synthetic peptides representing the juxtamembrane sequence of the receptor dose-dependently inhibited the binding of Src family tyrosine kinases to the autophosphorylated PDGF α-receptor. The mutant receptor showed similar PDGF-induced kinase activity and ability to mediate mitogenicity, actin reorganization and chemotaxis as the wild-type receptor. Thus activation of Src family kinases by the PDGF α-receptor is not essential for PDGF-induced mitogenicity or actin reorganization.

**SUMMARY**

Two novel autophosphorylation sites in the juxtamembrane region of the PDGF α-receptor, Tyr-572 and Tyr-574, were identified. A Y572/574F mutant PDGF α-receptor was generated and stably expressed in porcine aortic endothelial cells. In contrast to the wild-type receptor, the mutant receptor was unable to associate with or activate Src family tyrosine kinases. Tyrosine phosphorylated synthetic peptides representing the juxtamembrane sequence of the receptor dose-dependently inhibited the binding of Src family tyrosine kinases to the autophosphorylated PDGF α-receptor. The mutant receptor showed similar PDGF-induced kinase activity and ability to mediate mitogenicity, actin reorganization and chemotaxis as the wild-type receptor. Thus activation of Src family kinases by the PDGF α-receptor is not essential for PDGF-induced mitogenicity or actin reorganization.

Key words: PDGF α-receptor, Src, Mitogenicity
Corporation). An oligonucleotide with the sequence 5′-GA TGGACA TGAA TTTA TTTA TGTGGAC-3′ was used to introduce the Y572/574F mutation and one with the sequence 5′-GA TGGGCACTGAAATTTTTTTGTTGGGAC-3′ was used to introduce the Y572F mutation. The mutation was confirmed by nucleotide sequencing. Wild-type and mutated cDNAs were inserted into the eukaryotic expression vector pcDNA3 (Invitrogen). The constructs were transfected into porcine aortic endothelial (PAE) cells (Miyazono et al., 1987), by electroporation and subsequently selected with 608 μg/ml G418. Wild-type and mutated PDGF-α-receptor cDNAs were co-transfected into porcine aortic endothelial (PAE) cells, whereafter the samples were separated by SDS-PAGE and transferred onto nitrocellulose membrane. For in situ tryptic digestion, the radioactively labeled PDGF-α-receptor was cut out after exposure to film, and incubated with 0.5% polyvinylpyrrolidone-40, 0.6% acetic acid for 30 minutes at 37°C. The filter pieces were rinsed three times with water and then incubated for 12 hours at 37°C with 400 μl of 50 mM ammonium bicarbonate containing 1 μg of modified sequencing grade trypsin (Promega Corporation). The supernatant was lyophilized, oxidized in performic acid for 1 hour on ice, again lyophilized, resuspended in 50 μl of ammonium bicarbonate, and incubated with 1 μg of trypsin for another 12 hours at 37°C. The samples were lyophilized, dissolved in a pH 1.9 buffer consisting of 88% formic acid-acetic acid-water (50:156:1794), and analyzed by two-dimensional phosphopeptide mapping on a cellulose plate. Using electroforetic separation at pH 1.9 in the first dimension, followed by ascending chromatography (isobutyric acid:pyridine:glacial acetic acid:H₂O:n-butanol, 65:5:3:2:29) in the second. Radioactive phosphopeptides were visualized by autoradiography. When necessary, phosphopeptides on plates were scraped off and then eluted with the same buffer as for the mapping. In the case of immunoprecipitation of tryptic fragments, lyophilized tryptic digests were dissolved in 50 mM ammonium bicarbonate and incubated for 2 hours at 4°C with SSD-2 antisera covalently coupled to Protein A-Sepharose beads. The beads were then washed three times in 50 mM ammonium bicarbonate, 0.05% Triton X-100, twice in 150 mM ammonium bicarbonate, 0.05% Triton X-100 and twice in distilled water. The immunoprecipitated peptides were then eluted with 1% diethylamine (pH 11.9) and coupled to a Sequelon-AA membrane (Milligen/Biosearch, Burlington, MA) according to the manufacturer’s instructions, and Edman degradation was run in an Applied Biosystems gas phase sequencer. For phosphoamino acid analysis, peptides were subjected to hydrolysis in 6 M hydrochloric acid for 1 hour at 110°C, followed by separation by two-dimensional electrophoresis on a cellulose plate, and analysis by autoradiography.

**[32P]orthophosphate labeling, phosphopeptide mapping, Edman degradation, and phosphoamino acid analysis**

The procedures were performed essentially according to the method of Renström et al. (1992). Briefly, serum-starved cells were labeled by incubation in phosphate-free Ham’s F-12 medium supplemented with 0.5% dialyzed fetal calf serum and 4 μCi/ml [32P]orthophosphate for 3 hours at 37°C. Cells were treated with 100 ng/ml PDGF BB in the same medium for 1 hour at 4°C, and lysed in a lysis buffer containing 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.5% Triton X-100, 0.5% deoxycholate, 5 mM EDTA, 100 μM Na₃VO₄, 1% Trasylol and 1 mM PMSF. Immunoprecipitation was performed using PDGFR-7 antisera, whereafter the samples were separated by SDS-PAGE and then transferred onto nitrocellulose membrane. For in situ tryptic digestion, the radioactively labeled PDGF-α-receptor was cut out after exposure to film, and incubated with 0.5% polyvinylpyrrolidone-40, 0.6% acetic acid for 30 minutes at 37°C. The filter pieces were rinsed three times with water and then incubated for 12 hours at 37°C with 400 μl of 50 mM ammonium bicarbonate containing 1 μg of modified sequencing grade trypsin (Promega Corporation). The supernatant was lyophilized, oxidized in performic acid for 1 hour on ice, again lyophilized, resuspended in 50 μl of ammonium bicarbonate, and incubated with 1 μg of trypsin for another 12 hours at 37°C. The samples were lyophilized, dissolved in a pH 1.9 buffer consisting of 88% formic acid-acetic acid-water (50:156:1794), and analyzed by two-dimensional phosphopeptide mapping on a cellulose plate. Using electrophoretic separation at pH 1.9 in the first dimension, followed by ascending chromatography (isobutyric acid:pyridine:glacial acetic acid:H₂O:n-butanol, 65:5:3:2:29) in the second. Radioactive phosphopeptides were visualized by autoradiography. When necessary, phosphopeptides on plates were scraped off and then eluted with the same buffer as for the mapping. In the case of immunoprecipitation of tryptic fragments, lyophilized tryptic digests were dissolved in 50 mM ammonium bicarbonate and incubated for 2 hours at 4°C with SSD-2 antisera covalently coupled to Protein A-Sepharose beads. The beads were then washed three times in 50 mM ammonium bicarbonate, 0.05% Triton X-100, twice in 150 mM ammonium bicarbonate, 0.05% Triton X-100 and twice in distilled water. The immunoprecipitated peptides were then eluted with 1% diethylamine (pH 11.9) and coupled to a Sequelon-AA membrane (Milligen/Biosearch, Burlington, MA) according to the manufacturer’s instructions, and Edman degradation was run in an Applied Biosystems gas phase sequencer. For phosphoamino acid analysis, peptides were subjected to hydrolysis in 6 M hydrochloric acid for 1 hour at 110°C, followed by separation by two-dimensional electrophoresis on a cellulose plate, and analysis by autoradiography.

**[3H]thymidine incorporation assay**

The ability of PDGF to stimulate DNA synthesis in the different transfected cell lines, measured by the incorporation of [3H]thymidine, was performed as previously described (Mori et al., 1993).

**Actin reorganization**

The assay was a modification of the procedure described by Wennström et al. (1992). Briefly, cells cultured on glass coverslips in six-well plates were serum-starved and thereafter stimulated or not with 20 ng/ml PDGF-BB for 5 minutes at 37°C. The cells were fixed in 2% paraformaldehyde and permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature. The cells were rinsed three times with PBS and incubated with TRITC-labeled phalloidin (Sigma).
for 30 minutes. The cells were again rinsed three times with PBS and mounted in Fluoromount-G (Southern Biotechnology).

Chemotaxis assay

The chemotactic responses of wild-type and Y572/574F mutant α-receptor expressing PAE cells were measured using a Boyden chamber as described (Auerbach et al., 1991). A Costar nucleopore filter (8 μm thick, 8 μm pore) was coated with type-1 collagen (100 μg/ml) and dried. The lower chambers were filled with Ham’s F12 medium containing 0.1% fetal calf serum (FCS) supplemented with increasing concentrations of PDGF-BB. Ham’s F12 containing 10% FCS was used as a control. The precoated filter was then placed between the lower and upper chambers. Cultured cells were harvested and resuspended at a final concentration of 0.5×10⁶ cells/ml in Ham’s F12 containing 0.1% FCS. This cell suspension was added to the upper chambers and incubated at 37°C for 4 hours. Thereafter the filter was fixed in 99% ethanol and stained with Giemsa solution. Cells that had migrated through the filter were counted.

RESULTS

Characterization of a PAE cell line expressing a Y572/574F mutant of the PDGF α-receptor

In the PDGF β-receptor, two tyrosine residues in the juxtamembrane domain, Tyr-579 and Tyr-581, become phosphorylated upon ligand stimulation and are involved in binding Src family members (Mori et al., 1993). The juxtamembrane region of the PDGF α- and β-receptors are structurally similar (Fig. 1). To investigate the role of the corresponding tyrosine residues in the PDGF α-receptor, Tyr-572 and Tyr-574 codons of the cDNA encoding the wild-type human PDGF α-receptor were changed to phenylalanine codons by site-directed mutagenesis, generating the Y572/574F mutant receptor. The wild-type as well as the Y572/574F PDGF α-receptor mutant receptors were stably introduced into porcine aortic endothelial (PAE) cells.

In order to estimate the expression levels of the receptors, PAE cells expressing wild-type or mutant receptors were stimulated or not with PDGF, lysed and glycoproteins collected using wheat germ lectin Sepharose 6 MB. The samples were separated by SDS-PAGE, transferred onto nitrocellulose membrane and immunoblotted with PDGFR-7 (PDGF α-receptor anti-serum). As shown in Fig. 2A, the wild-type and Y572/574F mutant PDGF α-receptors were expressed to similar extents. The same filter was stripped and reprobed with the phosphotyrosine antibody PY20. Fig. 2B shows that ligand-binding stimulated autophosphorylation of wild-type and mutant receptors to similar degrees.

That the Y572/574F mutant PDGF α-receptor has intact kinase activity was shown by in vitro kinase assay performed on immunoprecipitated receptors (Fig. 2C). In contrast, the corresponding mutant of the PDGF β-receptor, Y579/581F, was found to have markedly decreased kinase activity in comparison to the wild-type β-receptor (Fig. 2C), consistent with previous observations (Mori et al., 1993; Vaillancourt et al., 1995).

Tyr572 and Tyr574 are in vivo phosphorylation sites in the PDGF α-receptor

We next examined the in vivo phosphorylation of Tyr-572 and Tyr-574 in response to PDGF stimulation. PAE cells expressing the wild-type, Y572F or Y572/574F mutant PDGF α-receptors were labeled with [32P]orthophosphate, incubated with PDGF, lysed and immunoprecipitated with PDGFR-7. The immunoprecipitated material was separated by SDS-PAGE, transferred onto nitrocellulose membrane and exposed to X-ray film. Thereafter, the phosphorylated PDGF α-receptor band

Fig. 2. Y572/574F mutant PDGF α-receptor undergoes autophosphorylation. (A) PAE cells expressing wild-type or Y572/574F mutant PDGF α-receptors were incubated with (+) or without (−) 100 ng/ml of PDGF, lysed and precipitated using wheat germ lectin Sepharose. The samples were separated by SDS-PAGE, transferred onto nitrocellulose membrane and analyzed by immunoblotting with anti-receptor antisemum (PDGFR-7). The position of the PDGF α-receptor is indicated (αR). (B) The blot shown in A was stripped, and reprobed with phosphotyrosine antibody PY20. (C) PAE cells expressing wild-type PDGF α-receptor, Y572/574F mutant PDGF α-receptor, wild-type β-receptor or Y579/581F mutant PDGF β-receptor were treated as in A and cell lysates were incubated with anti-receptor antisemum and subjected to in vitro kinase assay. The samples were separated by SDS-PAGE and then analyzed by autoradiography. ip, immunoprecipitation; ib, immunoblotting.
was excised from the membrane, subjected to in situ trypic digestion and the digest was analyzed by two-dimensional phosphopeptide mapping (Fig. 3). Phosphopeptide maps of the wild-type and the Y572/574F mutant receptor were similar except for a single phosphopeptide spot missing in the mutant receptor map (indicated by arrowheads). The data suggest that the mutant receptor lacks at least one phosphorylation site. To further confirm that the missing spot corresponds to a fragment of the juxtamembrane domain of the receptor, trypic digests of [32P]orthophosphate-labeled wild-type and mutant receptors were immunoprecipitated with an antiserum (SSD-2) raised against a synthetic peptide corresponding to the human PDGF β-receptor amino acid residues 573-586, a region which is highly similar to amino acid residues 566-580 in the PDGF α-receptor (Fig. 1). The immunoprecipitated material was thereafter subjected to Edman degradation, and the 32P radioactivity in the generated fragments was measured in each cycle. As shown in Fig. 4A, most of the 32P radioactivity was collected at cycles 12 and 14 in the wild-type PDGF α-receptor, corresponding to Tyr-572 and Tyr-574, respectively. As expected, the radioactive peak at cycle 12 corresponding to Tyr-572 was drastically diminished in the Y572F mutant whereas the peak at cycle 14 remained intact (Fig. 4B). In contrast, no radioactivity was detected in either of these cycles in the fragment derived from the Y572/574F mutant (Fig. 4C). Together with the phosphopeptide mapping results, we conclude that Tyr-572 and Tyr-574 are in vivo phosphorylation sites in the PDGF α-receptor.

**Tyr-572 and Tyr-574 in the PDGF α-receptor are binding sites for the Src family tyrosine kinases**

In the PDGF β-receptor, phosphorylated Tyr-579 and Tyr-581 in the juxtamembrane domain serve as binding sites for Src family tyrosine kinases (Mori et al., 1993). We therefore next investigated the ligand-induced interaction between Src family tyrosine kinases and wild-type and Y572/574F mutant PDGF α-receptors. The cells were treated as described above and the lysates were immunoprecipitated with an antiserum (cst.1) which recognizes the Src family members. The immunoprecipitates were separated and then analyzed by
immunoblotting with phosphotyrosine antibody PY20, to examine co-precipitation of the receptors. As shown in Fig. 5A, the association of Src molecules to the Y572/574F mutant receptor was severely reduced compared to that of the wild-type receptor.

In order to examine the involvement in binding Src family members of Tyr-572 and Tyr-574 individually, inhibition of complex formation was performed using phosphorylated peptides. Peptides corresponding to the juxtamembrane region of the human PDGF α-receptor were synthesized, in which Tyr-572 and Tyr-574 were phosphorylated individually or simultaneously (Table 1). PAE cells expressing the wild-type PDGF α-receptor were treated with PDGF, and the cell lysate was incubated with the synthetic peptides for 1 hour prior to immunoprecipitation with cst.1 anti-serum. The samples were separated and analyzed by PY20 immunoblotting. As shown in Fig. 5B, phosphorylated peptides inhibited complex formation between Src family tyrosine kinases and wild-type PDGF α-receptor dose-dependently. The efficiency of inhibition using 10 μM peptide was highest in the doubly phosphorylated (pY572/pY574) peptide; the pY572 peptide also efficiently inhibited complex formation, whereas the pY574 peptide was less efficient. Thus, phosphorylated Tyr-572 and Tyr-574 both serve as binding sites for Src family members.

**Table 1. Synthetic peptides used for the inhibition experiment**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Amino acid sequence</th>
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<tbody>
<tr>
<td>Reference</td>
<td>I-S-P-D-G-H-E-Y - I - Y -V-D-P-M-Q-L-P-Y</td>
</tr>
<tr>
<td>pY572/pY574</td>
<td>I-S-P-D-G-H-E-pY - I - pY -V-D-P-M-Q-L-P-Y</td>
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</tbody>
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Activation of Src family tyrosine kinases is abolished in the Y572/574F mutant PDGF α-receptor

PDGF-induced activation of Src family tyrosine kinases was examined using enolase as an exogenous substrate. Cst.1 immunoprecipitates from PAE cells expressing the wild-type or the Y572/574F mutant PDGF α-receptor were subjected to in vitro kinase assay in the presence of enolase, followed by separation and analysis by autoradiography. Incorporation of 32P radioactivity into enolase, as well as into Src itself, increased upon PDGF-stimulation in the wild-type receptor but not in the Y572/574F mutant (Fig. 6). Thus, binding to ligand-stimulated receptor is a prerequisite for Src kinases to be activated in PDGF α-receptor mediated signaling pathways, measured as phosphorylation of Src itself as well as phosphorylation of an exogenous substrate.

**PDGF-BB-stimulated [3H]thymidine incorporation and actin reorganization in cells expressing wild-type or Y572/574F mutant PDGF α-receptor**

The ability of the wild-type and mutant receptors to transduce mitogenic signals was examined by [3H]thymidine incorporation assay (Fig. 7). Two clones each of wild-type and Y572/574F mutant receptor expressing cells, representative of several other clones, are shown. Both types of receptors were able to respond to stimulation by PDGF with a similar dose-dependency. Non-transfected PAE cells do not respond to PDGF with increased [3H]thymidine incorporation (Rönnstrand et al., 1992).

**Stimulation of PAE cells expressing the PDGF α-receptor with PDGF induces reorganization of the actin filament system.** This includes the appearance of veil-like actin-containing structures around the edges of the cell, i.e. edge ruffles, and a loss of actin stress fibers, visualized as bundles of actin spanning the cytoplasm. Upon PDGF stimulation, PAE cells expressing the Y572/574F mutant receptor showed a
similar rearrangement of the actin filament system as cells with wild-type receptors (Fig. 8). Non-transfected PAE cells have previously been shown not to respond to PDGF in actin reorganization assays (Eriksson et al., 1992). Therefore, Src appears not to play a crucial role in PDGF-mediated mitogenesis or reorganization of the actin cytoskeleton.

**DISCUSSION**

In this study, we have identified two novel autophosphorylation sites, Tyr-572 and Tyr-574, in the juxtamembrane region of the PDGF α-receptor and shown that these sites are required for the binding and activation of Src family tyrosine kinases. We found that the Y572/574F mutant PDGF α-receptor, despite the lack of association to and activation of Src family tyrosine kinases, is fully capable of responding mitogenically to PDGF.

Tyr-579 and Tyr-581 have previously been shown to be autophosphorylation sites in the PDGF β-receptor and to serve as binding sites for Src family tyrosine kinases (Mori et al., 1993). However, the autophosphorylation of the PDGF β-receptor was dramatically decreased when both of these tyrosine residues were replaced by phenylalanine residues (Mori et al., 1993; Vaillancourt et al., 1995). Thus, it has not been possible to definitely elucidate the role of Src family kinases in PDGF signaling using PDGF β-receptor Src-binding mutants. In contrast, the autophosphorylation of the Y572/574F mutant PDGF α-receptor was similar to that of the wild-type receptor (Fig. 2C).

Microinjection studies have shown that Src family kinases are involved in mitogenic signaling of PDGF receptors (Barone and Courtneidge, 1995; Twamley-Stein et al., 1993). In our study, the Y572/574F mutant PDGF α-receptor, which is unable to bind to or activate Src family tyrosine kinases, still mediated DNA synthesis upon ligand stimulation. A possibility which remains to be elucidated is that Src family members are activated downstream in signaling pathways initiated at PDGF receptors. Recently, Myc has been proposed as a downstream mediator of Src-dependent, PDGF-mediated, mitogenic signaling (Barone and Courtneidge, 1995; Eisenman and Cooper, 1995). Due to relatively high background levels of c-myc mRNA in serum-starved PAE cells it was difficult to detect an induction upon PDGF-stimulation (data not shown). Therefore the role of Src in PDGF-induced c-myc induction in PAE cells remains to be clarified.

Src has been shown to be able to activate members of the Raf-1 family of Ser/Thr kinases (Marais et al., 1995; Stokoe and McCormick, 1997), which in turn activate MEK and MAP
kinases. The Y572/574F mutant was, however, found to be able to induce MAP kinase activity upon PDGF stimulation to a degree similar to the wild-type PDGF α-receptor (data not shown). In v-Src transformed cells, the adaptor protein Shc is tyrosine phosphorylated (McGlade et al., 1992), and may thus mediate Ras activation via recruitment of the Grb2-Sos complex (Egan et al., 1993). It has also been shown that transformation by v-Src could be reversed by inhibiting Ras function (Smith et al., 1986). Therefore, we considered the possibility that the Src family kinases are involved in PDGF-induced Ras activation via phosphorylation of Shc. However, the phosphorylation level of Shc was found to be similar in PAE cells expressing the wild-type and Y572/574F mutant PDGF α-receptor (data not shown), which is in line with the adequate level of MAP kinase activation and mitogenic response of the mutant receptor.

Members of the Src family have been shown to associate with and phosphorylate PLC-γ in vitro (Liao et al., 1993; Nakaniishi et al., 1993). We therefore investigated the phosphorylation and activation of PLC-γ in the Y572/574F mutant PDGF α-receptor expressing cells. We observed that PLC-γ phosphorylation levels were comparable in the wild-type and mutant PDGF α-receptors (data not shown). This suggests that binding of PLC-γ to Tyr-1018 and Tyr-988 in the PDGF α-receptor (Eriksson et al., 1995) is sufficient for phosphorylation, and that this phosphorylation takes place independent of Src kinase activity in response to PDGF stimulation.

Src kinases have been reported to activate PI3-kinase (Cantley et al., 1991) which is involved in PDGF-mediated actin reorganization and chemotaxis (Wenström et al., 1994). The C-terminal Src kinase (Csk) phosphorylates Src family tyrosine kinases and downregulates their activity in vitro (Okada et al., 1991). Elevated Src activity in Csk-deficient cells leads to impaired organization of actin stress fibers, and decreased cell attachment (Nada et al., 1994). However, no significant difference in PDGF-induced actin reorganization in PAE cells expressing wild-type or Y572/574F mutant PDGF α-receptors was found (Fig. 8), nor was the chemotactic response to PDGF perturbed in this mutant (data not shown), implicating that Src is not critical in PDGF α-receptor mediated actin reorganization and chemotaxis.

In conclusion, we have found two new autophosphorylation sites in the PDGF α-receptor which are responsible for Src binding upon PDGF stimulation. Src binding to the PDGF α-receptor, however, seems not to be necessary for receptor kinase activity, nor for the ability of the receptor to recruit and phosphorylate other substrates such as Shc and PLC-γ. The intact mitogenic response of the Y572/574F mutant receptor is consistent with the finding that MAP kinase activity is undisturbed in this mutant. Future studies will be aimed at determining the precise role of Src in PDGF α-receptor signaling. An important tool in these studies would be gene targeted cells lacking expression of Src, Fyn and Yes. The use of such cells would help answer the question of whether Src family members act further down-stream in PDGF-mediated signal transduction pathways.

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