Stimulation of the α_{1A} adrenergic receptor inhibits PDGF-induced PDGF β receptor Tyr751 phosphorylation and PI 3-kinase activation

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Abstract **Several reports indicate that some $G_{\alpha q}$-coupled receptors antagonize the activation of phosphatidylinositol (PI) 3-kinase by receptor tyrosine kinases. We used Rat-1 fibroblasts expressing the α_{1A} adrenergic receptor to study how this $G_{\alpha q}$-coupled receptor inhibits platelet-derived growth factor (PDGF) activation of PI 3-kinase. Phenylephrine (PE) stimulation of the α_{1A} adrenergic receptor inhibited PDGF-induced binding of PI 3-kinase to the PDGF receptor (PDGFR) and phosphorylation of the PDGFR at Tyr751, which forms a docking site for PI 3-kinase. By contrast, activation of phospholipase Cγ by PDGF and phosphorylation of the PDGFR at Tyr716 and Tyr771 were not inhibited by PE. The protein tyrosine phosphatase SHP-2, which dephosphorylates Tyr751 on the PDGFR, was more active in cells treated with PDGF plus PE than in cells treated with either agent alone. PDGF-induced PI 3-kinase signaling was also inhibited by treatment of cells with *Pasteurella multocida* toxin to activate $G_{\alpha q}$. These results suggest that the α_{1A} adrenergic receptor, and perhaps other $G_{\alpha q}$-coupled receptors, uses tyrosine dephosphorylation to block PI 3-kinase activation by PDGF.**

Key words: Phosphatidylinositol 3-kinase; G_q-coupled receptor; Akt; SHP-2; *Pasteurella multocida* toxin

1. Introduction

Phosphatidylinositol (PI) 3-kinase mediates many of the cellular actions of receptor tyrosine kinases [1]. Class IA PI 3-kinases are heterodimers between a p110 catalytic subunit and a p85 or p55 regulatory subunit. The p85 subunit contains two Src homology (SH) 2 domains that bind to specific phosphotyrosine motifs on receptor tyrosine kinases or their substrates. For example, phospho-Tyr740 and phospho-Tyr751 on the platelet-derived growth factor (PDGF) β receptor (PDGFRβ) form a high-affinity binding site that recruits the p110/p85 heterodimer to the activated receptor [2]. This interaction leads to translocation of p110 to the membrane and enhances its catalytic activity.

The serine/threonine protein kinase Akt is a major downstream effector of PI 3-kinase [3]. Phosphoinositides generated by PI 3-kinase bind to the pleckstrin homology domain of Akt, inducing a conformational change that allows Akt to be phosphorylated and activated. Substrates of Akt include glycogen synthase kinase 3, whose phosphorylation and inactivation is thought to contribute to insulin-stimulated glycogen synthesis, and BAD, which loses its apoptotic function upon phosphorylation by Akt [3]. Negative regulation of PI 3-kinase/Akt signaling can therefore have profound effects on glucose metabolism and cell survival.

We recently reported that stimulation of the α_{1A} adrenergic receptor, a serpentine receptor that signals through heterotrimeric G proteins, inhibits PDGF activation of PI 3-kinase and Akt in Rat-1 fibroblasts [4]. In this study, we investigated mechanisms by which the α_{1A} adrenergic receptor might mediate this response. We present evidence that the α_{1A} adrenergic receptor attenuates PDGF-dependent tyrosine phosphorylation of the PDGFR and recruitment of PI 3-kinase to the activated receptor.

2. Materials and methods

2.1. Materials

Human recombinant PDGF A/B and phenylephrine (PE) were purchased from Sigma. [γ-^{32}P]ATP (3000 Ci/mmol) was from Perkin Elmer Life Sciences. PI was purchased from Avanti Polar Lipids. PY20 phosphotyrosine antibody was from Transduction Laboratories. Rabbit polyclonal antibodies against the PDGFRβ, phospholipase C (PLC)γ1, SHP-2, and Akt (H-136) were from Santa Cruz Biotechnology, Santa Cruz, CA, USA. p85α monoclonal antibody and phospho-Tyr716 PDGFRβ antibody were from Upstate Biotechnology, Lake Placid, NY, USA. Phospho-Ser473 Akt antibody was from Cell Signaling Technology, and *Pasteurella multocida* toxin (PMT) was from Calbiochem, San Diego, CA, USA. Rabbit antibodies to phospho-Tyr771 PDGFRβ were raised using the peptide KYADIESSN(pY)-MAPYDK as an immunogen and were affinity-purified (Alpha Diagnostic International, San Antonio, TX, USA).

2.2. Cell culture and lysate preparation

Rat-1 fibroblasts stably expressing the human α_{1A} adrenergic receptor [5] were maintained in Dulbecco's modified Eagle's medium (Mediatech) with antibiotics and 10% fetal bovine serum (Sigma). The cells were incubated overnight in serum-free medium before agonist treatments. After treatment, cells were rinsed with ice-cold phosphate-buffered saline and scraped into lysis buffer (50 mM Tris, pH 7.5, 120 mM NaCl, 1% Triton X-100, 1 mM EDTA, 50 mM NaF, 40 mM 2-glycerophosphate, 0.1 mM sodium orthovanadate, 1 mM benzami-

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Abbreviations: PI, phosphatidylinositol; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; PE, phenylephrine; PLC, phospholipase C; PMT, *Pasteurella multocida* toxin; SH, Src homology; IGF-I, insulin-like growth factor I; IRS-1, insulin receptor substrate-1

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dine, 0.5 mM phenylmethylsulfonyl fluoride, and 10 μg/ml aprotinin and leupeptin) on ice.

Homogenates were centrifuged at 15 000×*g* for 15 min at 4°C, and protein concentrations were determined using a Bradford assay (Bio-Rad).

2.3. Immunoprecipitation and Western blotting

Cell lysates containing equal amounts of protein were incubated with the appropriate antibody for 2 h or overnight at 4°C, and then with 12.5 μl of protein A or G agarose (Sigma) for 1 h. The beads were either washed three times with lysis buffer and used for immunoblotting, or they were washed three times with lysis buffer and once with the appropriate kinase assay buffer prior to performing kinase assays. Immunoprecipitates or equal amounts of cell lysate protein were subjected to SDS–polyacrylamide gel electrophoresis followed by electrophoretic transfer onto nitrocellulose or polyvinylidene difluoride membranes. Signals were visualized using horseradish peroxidase-linked secondary antibodies (Amersham Pharmacia Biotechnology) and a chemiluminescence kit (Perkin Elmer Life Sciences). Blots were stripped as described earlier [4].

2.4. PI 3-kinase assay

PI 3-kinase activity was assayed following methods described previously [4].

2.5. SHP-2 assay

^{32}P-labeled myelin basic protein was prepared using the Protein Tyrosine Phosphatase Assay System kit from New England Biolabs. Rat-1 cells in 10 cm dishes were serum-starved overnight after reaching approximately 80% confluence. After the treatments described in the text, the cells were washed with cold phosphate-buffered saline and scraped into 1 ml of extraction buffer (50 mM Na_2HPO_4, 120 mM NaCl, 1% Triton X-100, 20 mM *p*-nitrophenyl phosphate, 2 mM EDTA, 1 mM dithiothreitol, 20 mM NaF, 0.2 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 1 μg/ml pepstatin, 1.7 μg/ml aprotinin, and 1 mM benzamidine, pH 7.0). After centrifuging for 10 min at 15 000×*g* at 4°C, equal amounts of cell lysate protein were incubated with 1 μg of SHP-2 antibody for 3 h on ice, and then with 12.5 μl of protein A agarose for 1 h. The beads were washed three times with extraction buffer and once with phosphatase assay buffer (New England Biolabs) containing only 1 mM dithiothreitol. Phosphatase assays (50 μl) contained 5 μl of ^{32}P-labeled myelin basic protein and 1 mg/ml bovine serum albumin in assay buffer. After 30 min at 37°C, the assays were stopped and the ^{32}P*i* released was determined according to the kit instructions.

2.6. PDGFR autophosphorylation assay

Washed PDGFRβ immunoprecipitates were suspended in 25 μl of kinase assay buffer (50 mM Tris, pH 7.5, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM benzamidine, and 0.5 mM phenylmethylsulfonyl fluoride) containing 10 μCi of [γ-^{32}P]ATP. After incubation for 30 min at 37°C, the reactions were terminated by heating with SDS sample buffer and the samples were subjected to SDS–polyacrylamide gel electrophoresis.

3. Results

3.1. PE inhibits PDGF-induced PI 3-kinase activation

We showed earlier that PE stimulation of the α_{1A} adrenergic receptor in Rat-1 cells inhibited PDGF-induced activation of PI 3-kinase, as measured in phosphotyrosine immunoprecipitates [4]. To further examine the effect of the α_{1A} adrenergic receptor on PI 3-kinase activation, we assayed PI 3-kinase activity associated with the PDGFR. Rat-1 cells were treated with PDGF in the presence or absence of PE, and cell extract proteins were immunoprecipitated with a PDGFRβ antibody. As expected, PI 3-kinase activity in the immunoprecipitates was greatly increased following treatment of cells with PDGF, but no increase was detected after exposure to PE alone (Fig. 1). The PDGF-induced activation of PI 3-kinase was reduced by 43% in the presence of PE (Fig. 1).

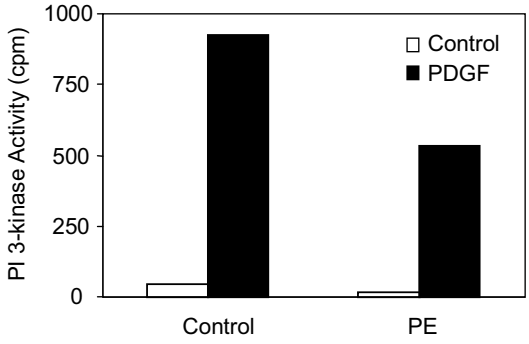

Fig. 1. Effect of PE on PDGFR-associated PI 3-kinase activity. Cells were stimulated for 5 min with 25 ng/ml PDGF in the presence or absence of 10 μM PE. PI 3-kinase activity was measured in PDGFRβ immunoprecipitates. The experiment was repeated with similar results.

3.2. PE inhibits tyrosine phosphorylation of the PDGFR and binding to PI 3-kinase

One explanation for the result in Fig. 1 is that PE causes a reduction in binding of PI 3-kinase to the activated PDGFR. To test this possibility, we examined the association of the PI 3-kinase p85α regulatory subunit with the PDGFRβ. The amount of p85α that coimmunoprecipitated with the PDGFR was markedly reduced in cells treated with PDGF plus PE, as compared to cells treated with PDGF alone (Fig. 2A, top blot). This result would be obtained if tyrosine phosphorylation of the PDGFR is reduced in the presence of PE. To test this possibility, the blot was reprobed with an antibody to phosphotyrosine. Tyrosine phosphorylation of the PDGFR was lower in cells treated with PDGF plus PE than in cells treated with PDGF alone (Fig. 2A, middle blot). The blot was reprobed with PDGFRβ antibody to show that similar amounts of the receptor were present in each lane (Fig. 2A, lowest blot). Similarly, the amount of p85α and tyrosine-phosphorylated PDGFRβ in anti-phosphotyrosine immunoprecipitates was lower in cells treated with PDGF plus PE than in cells treated with PDGF alone (Fig. 2B).

We next used a phosphospecific antibody [6] to detect changes in the phosphorylation state of the p85 binding site at Tyr751 on the PDGFRβ. Consistent with the previous results, phosphorylation of Tyr751 increased in cells treated with PDGF, and this response was reduced in the presence of PE (Fig. 2C, upper blot). The blot was reprobed with PDGFRβ antibody to demonstrate equivalent loading of the protein in each lane (Fig. 2C, lower blot). These results show that activation of the α_{1A} adrenergic receptor antagonizes ligand-induced phosphorylation of the PDGFR at Tyr751, resulting in reduced binding of PI 3-kinase to the receptor.

3.3. Specificity of PE inhibition of PDGFR signaling

Ligand binding to the PDGFR promotes autophosphorylation of multiple tyrosine residues that recruit diverse signaling molecules [7]. Attenuated PDGFR tyrosine phosphorylation in the presence of PE could be due to a decrease in autokinase activity of the PDGFR or to activation of PDGFR phosphatases. If PE treatment causes a decrease in autokinase activity, then one might expect that other PDGF-activated signaling pathways in addition to PI 3-kinase would be attenuated. It is thought that PLCγ is recruited to and phosphorylated by the PDGFR in response to PDGF stimulation [8]. To test if PE

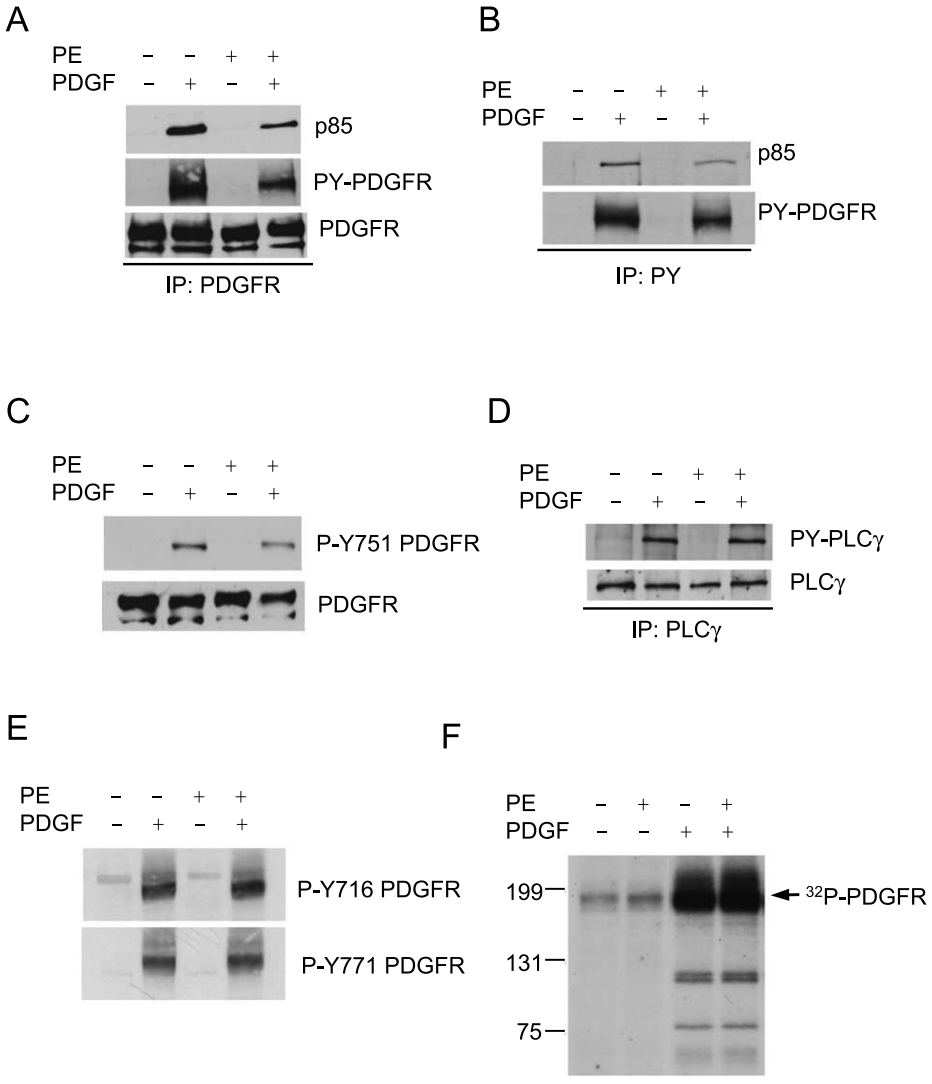

Fig. 2. Effect of PE on binding of PI 3-kinase to the PDGFR. Cells were treated for 5 min with 50 ng/ml PDGF in the presence or absence of 10 μM PE. The results shown are representative of three independent experiments. PY: Phosphotyrosine; IP: immunoprecipitation. A: PDGFRβ immunoprecipitates were analyzed on a Western blot probed sequentially with antibodies to p85α (top blot), phosphotyrosine (middle blot), and the PDGFRβ (lowest blot). B: Phosphotyrosine immunoprecipitates were analyzed on a Western blot probed sequentially with antibodies to p85α (upper blot) and phosphotyrosine (lower blot). C: Cell lysate proteins were analyzed on a Western blot probed sequentially with antibodies to phospho-Tyr751 PDGFRβ (upper blot) and total PDGFRβ (lower blot). D: PLCγ immunoprecipitates were analyzed on a Western blot probed sequentially with antibodies to phosphotyrosine (upper blot) and PLCγ (lower blot). E: Cell lysate proteins were analyzed on Western blots probed with antibodies to phospho-Tyr716 PDGFRβ (upper blot) and phospho-Tyr771 PDGFRβ (bottom blot). F: PDGFRβ immunoprecipitates were subjected to an autophosphorylation assay (see Section 2). ^{32}P-labeled proteins were visualized by autoradiography. Numbers in kDa indicate the position of protein standards.

inhibits PDGF-induced tyrosine phosphorylation of PLCγ, Rat-1 cells were treated with PDGF in the presence or absence of PE, and cell extract proteins were immunoprecipitated with a PLCγ antibody. The immunoprecipitates were then probed on a Western blot with antibody to phosphotyrosine. As expected, PDGF treatment stimulated the robust tyrosine phosphorylation of PLCγ (Fig. 2D, upper blot). However, cotreatment with PE did not reduce the level of tyrosine phosphorylation of this protein. The blot was reprobed with PLCγ antibody to show that similar amounts of PLCγ were present in each lane (Fig. 2D, lower blot). Similarly, phosphorylation of Tyr716 and Tyr771 on the PDGFRβ was not reduced in the presence of PE (Fig. 2E).

Finally, PDGFR kinase activity was measured in PDGFRβ immunoprecipitates. As expected, the PDGFR from cells stimulated with PDGF had a markedly increased ability to autophosphorylate in vitro as compared with the receptor from untreated control cells (Fig. 2F). Treatment with PE plus PDGF did not reduce PDGFR autophosphorylation. These results indicate that stimulation of the α_{1A} adrenergic receptor does not inhibit all PDGFR-mediated responses, and the effect on phosphorylation of Tyr751 is probably not due to a decrease in PDGFR kinase activity.

3.4. Activation of SHP-2 by PE plus PDGF

We next asked whether activation of a protein tyrosine phosphatase could account for reduced phosphorylation of the PDGFRβ at Tyr751. SHP-2 is a ubiquitously expressed

protein tyrosine phosphatase that contains two SH2 domains. SHP-2 binds to phospho-Tyr1009 on the activated PDGFRβ and may participate in the downregulation of receptor signaling by dephosphorylating selective docking sites, including phospho-Tyr751 and phospho-Tyr740 [9,10]. We measured protein tyrosine phosphatase activity in SHP-2 immunoprecipitates after treating cells with growth factors alone or in combination. Although there was a trend toward higher SHP-2 phosphatase activity in response to either PDGF or PE alone, the difference as compared to untreated control cells was not statistically significant (Fig. 3). However, after treatment with both agents together there was a 60% increase in activity that was highly statistically significant (Fig. 3). This result suggests that activation of SHP-2 and subsequent dephosphorylation of Tyr751 on the PDGFRβ could account at least in part for the inhibitory effect of the α_{1A} adrenergic receptor on PDGF-dependent PI 3-kinase signaling.

3.5. PMT inhibits PDGFR phosphorylation and signaling to Akt

Stimulation of G protein-coupled receptors leads to GTP loading of the heterotrimeric G protein α subunit and release of the βγ subunits. The α and βγ proteins then exert their effects on downstream effectors. Although the α_{1A} adrenergic receptor couples mainly to G_α proteins in the q/11 family, the receptor can also activate other types of G_α subunits [11]. Since $G_{\beta\gamma}$ subunits activate PI 3-kinase [12,13], we decided to explore a possible role for $G_{\alpha q}$ in PI 3-kinase inhibition. Treatment of cells with PMT induces a number of responses, including activation of Rho, increased hydrolysis of phosphoinositides, mobilization of intracellular Ca^{2+}, and activation of protein kinase C. The latter three effects appear to be due to the activation of $G_{\alpha q}$ [14,15]. Rat-1 cells were treated with or without PMT prior to stimulation with PDGF, and the phosphorylation state of Tyr751 on the PDGFRβ was analyzed. The amount of phospho-Tyr751 was reduced in cells pretreated with PMT as compared with cells treated with PDGF in the absence of the toxin (Fig. 4, top blot). The inhibitory effect of PMT on Tyr751 phosphorylation was stronger than that seen using PE (Fig. 2C). To determine the effect of PMT on PI 3-kinase signaling, we examined the phosphorylation state of Akt. Western analysis using a phosphospecific antibody showed that PMT pretreatment partly inhibited PDGF-induced Akt phosphorylation at Ser473 (Fig. 4, third blot). PMT treatment did not affect the expression of the PDGFR or Akt (Fig. 4, second and bottom blots). Together, these results suggest that activation of $G_{\alpha q}$, either by agonists that bind to G_q-coupled receptors or by treatment of cells with PMT, inhibits PDGF-dependent PI 3-kinase/Akt signaling.

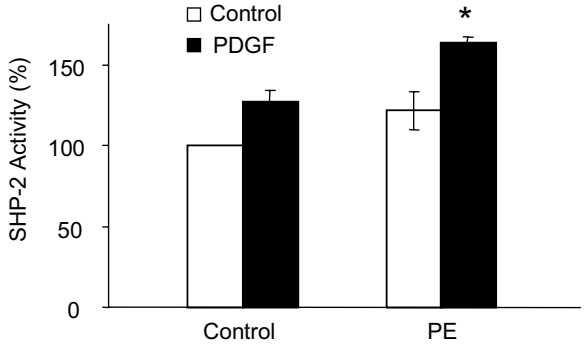

Fig. 3. Effect of growth factors on SHP-2 activity. Cells were stimulated for 5 min with 25 ng/ml PDGF in the presence or absence of 10 μM PE. Protein tyrosine phosphatase activity was measured in SHP-2 immunoprecipitates (see Section 2). The data shown are means ± standard error from four separate experiments. The asterisk designates a significant difference between Control and PE+PDGF ($P < 0.001$) and between PDGF and PE+PDGF ($P < 0.05$). Pairwise comparisons were made using Fisher's post hoc tests.

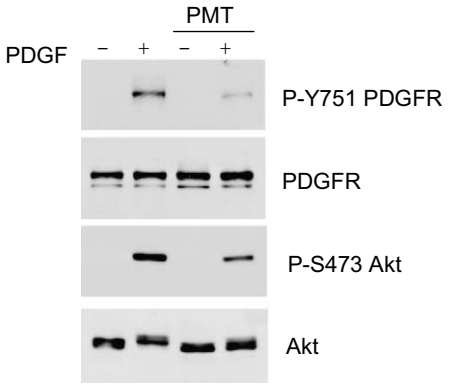

Fig. 4. Effect of PMT on PDGFR signaling. Cells were treated for 18 h with or without 400 ng/ml PMT prior to stimulation for 5 min with or without 25 ng/ml PDGF. Equal amounts of cell lysate protein were analyzed on Western blots probed sequentially with antibodies to phospho-Tyr751 PDGFRβ (top blot), total PDGFRβ (second blot), phospho-Ser473 Akt (third blot), and total Akt (bottom blot). The experiment was repeated with similar results.

4. Discussion

Coordinate regulation of PI 3-kinase activity by receptor tyrosine kinases and G_q-coupled receptors can substantially alter cellular responses such as survival. Activation of PI 3-kinase/Akt by receptor tyrosine kinases promotes cell survival [16], whereas activation of $G_{\alpha q}$ induces apoptosis in some cell types [4]. Cardiac myocytes from transgenic mice that express constitutively active $G_{\alpha q}$ show an increased rate of apoptosis [17], and G_q-coupled receptors are known to play an important role in the development of heart failure [18]. Interference with receptor tyrosine kinase activation of PI 3-kinase may explain how G_q-coupled receptors promote cell death. We have shown that PE treatment of Rat-1 cells expressing the α_{1A} adrenergic receptor augments UV-induced apoptosis and inhibits activation of PI 3-kinase/Akt in response to PDGF, insulin, and insulin-like growth factor I (IGF-I) [4,19]. This study explores one mechanism underlying this inhibitory effect.

Our results show that activation of the α_{1A} adrenergic receptor reduces PDGF-induced phosphorylation of the PDGFR at Tyr751, thus eliminating a docking site for the p85 subunit of PI 3-kinase. Decreased phosphorylation of the PDGFR may be due to an increased rate of dephosphorylation by SHP-2. It was reported that SHP-2 preferentially dephosphorylates sites on the PDGFRβ that are important for PI 3-kinase binding, whereas the docking site for PLCγ is a poor substrate [10]. Our finding that PE inhibits PDGF activation of PI 3-kinase but not PLCγ is consistent with those results. SHP-2 has been reported to be activated by tyrosine

phosphorylation [20] or by binding of phosphotyrosyl peptides to its SH2 domains [21], whereas serine/threonine phosphorylation [22] and reactive oxygen species [23] have been reported to inhibit the enzyme. We currently do not know if any of these mechanisms contribute to SHP-2 activation in cells treated with PDGF plus PE.

Some other G_q-coupled receptors have been shown to antagonize growth factor activation of PI 3-kinase by reducing tyrosine phosphorylation. For example, pretreatment of rat aortic smooth muscle cells with angiotensin II inhibited insulin-stimulated tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1), resulting in decreased PI 3-kinase activation [24]. It was hypothesized that either (a) increased serine phosphorylation of IRS-1 in the presence of angiotensin II prevents the protein from docking to and being phosphorylated by the activated insulin receptor, thus preventing IRS-1 from binding and activating PI 3-kinase, or (b) angiotensin II induces the rapid dephosphorylation of IRS-1 via the activation of a protein tyrosine phosphatase. We speculate that protein tyrosine dephosphorylation might be a general mechanism used by G_q-coupled receptors to oppose the activation of PI 3-kinase by a variety of growth factors.

Although PMT exerts pleiotropic effects on cells, recent studies in fibroblasts lacking $G_{\alpha q}$ or the closely related $G_{\alpha 11}$ showed that production of inositol phosphates in response to toxin treatment is dependent on $G_{\alpha q}$ (not $G_{\alpha 11}$) [15]. We used PMT to investigate how receptor-independent activation of $G_{\alpha q}$ affects PDGF signaling, and found that the toxin mimics the effect of PE by inhibiting PDGFRβ phosphorylation and Akt activation. PMT has also been shown to inhibit Akt phosphorylation promoted by epidermal growth factor or IGF-I treatment of neonatal rat cardiac myocytes [25]. Based on these results, we hypothesize that activated $G_{\alpha q}$ mediates PI 3-kinase inhibition. This hypothesis is supported by the observation that expression of an activated mutant of $G_{\alpha q}$ opposes growth factor activation of PI 3-kinase (L.M. Ballou, H.-Y. Lin, G. Fan, Y.-P. Jiang and R.Z. Lin, submitted manuscript).

In conclusion, our results demonstrate that one mechanism used by the α_{1A} adrenergic receptor to antagonize PDGF activation of PI 3-kinase involves dephosphorylation of the PDGFR. Further study of the cross-talk between these two classes of receptors might reveal important insights into diseases such as heart failure and diabetes.

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References

[1] Katso, R., Okkenhaug, K., Ahmadi, K., White, S., Timms, J. and Waterfield, M.D. (2001) Annu. Rev. Cell Dev. Biol. 17, 615–675.
[2] Kashishian, A., Kazlauskas, A. and Cooper, J.A. (1992) EMBO J. 11, 1373–1382.
[3] Alessi, D.R. and Cohen, P. (1998) Curr. Opin. Genet. Dev. 8, 55–62.
[4] Ballou, L.M., Cross, M.E., Huang, S., McReynolds, E.M., Zhang, B.X. and Lin, R.Z. (2000) J. Biol. Chem. 275, 4803–4809.
[5] Kenny, B.A., Miller, A.M., Williamson, I.J., O'Connell, J., Chalmers, D.H. and Naylor, A.M. (1996) Br. J. Pharmacol. 118, 871–878.
[6] Bernard, A. and Kazlauskas, A. (1999) Exp. Cell Res. 253, 704–712.
[7] Claesson-Welsh, L. (1994) J. Biol. Chem. 269, 32023–32026.
[8] Noh, D.Y., Shin, S.H. and Rhee, S.G. (1995) Biochim. Biophys. Acta 1242, 99–113.
[9] Kazlauskas, A., Feng, G.S., Pawson, T. and Valius, M. (1993) Proc. Natl. Acad. Sci. USA 90, 6939–6943.
[10] Klinghoffer, R.A. and Kazlauskas, A. (1995) J. Biol. Chem. 270, 22208–22217.
[11] Ruan, Y., Kan, H., Parmentier, J.H., Fatima, S., Allen, L.F. and Malik, K.U. (1998) J. Pharmacol. Exp. Ther. 284, 576–585.
[12] Stoyanov, B., Volinia, S., Hanck, T., Rubio, I., Loubtchenkov, M., Malek, D., Stoyanova, S., Vanhaesebroeck, B., Dhand, R., Nurnberg, B., Gierschik, P., Seedorf, K., Hsuan, J.J., Waterfield, M.D. and Wetzker, R. (1995) Science 269, 690–693.
[13] Kurosu, H., Maehama, T., Okada, T., Yamamoto, T., Hoshino, S., Fukui, Y., Ui, M., Hazeki, O. and Katada, T. (1997) J. Biol. Chem. 272, 24252–24256.
[14] Wilson, B.A., Zhu, X., Ho, M. and Lu, L. (1997) J. Biol. Chem. 272, 1268–1275.
[15] Zywietz, A., Gohla, A., Schmelz, M., Schultz, G. and Offermanns, S. (2001) J. Biol. Chem. 276, 3840–3845.
[16] Fujio, Y., Nguyen, T., Wencker, D., Kitsis, R.N. and Walsh, K. (2000) Circulation 101, 660–667.
[17] Adams, J.W., Sakata, Y., Davis, M.G., Sah, V.P., Wang, Y., Liggett, S.B., Chien, K.R., Brown, J.H. and Dorn, G.W. (1998) Proc. Natl. Acad. Sci. USA 95, 10140–10145.
[18] Hunter, J.J. and Chien, K.R. (1999) N. Engl. J. Med. 341, 1276–1283.
[19] Ballou, L.M., Tian, P.Y., Lin, H.Y., Jiang, Y.P. and Lin, R.Z. (2001) J. Biol. Chem. 276, 40910–40916.
[20] Vogel, W., Lammers, R., Huang, J. and Ullrich, A. (1993) Science 259, 1611–1614.
[21] Pluskey, S., Wandless, T.J., Walsh, C.T. and Shoelson, S.E. (1995) J. Biol. Chem. 270, 2897–2900.
[22] Peraldi, P., Zhao, Z., Filloux, C., Fischer, E.H. and Van Obberghen, E. (1994) Proc. Natl. Acad. Sci. USA 91, 5002–5006.
[23] Meng, T.C., Fukada, T. and Tonks, N.K. (2002) Mol. Cell 9, 387–399.
[24] Folli, F., Kahn, C.R., Hansen, H., Bouchie, J.L. and Feener, E.P. (1997) J. Clin. Invest. 100, 2158–2169.
[25] Sabri, A., Wilson, B.A. and Steinberg, S.F. (2002) Circ. Res. 90, 850–857.