Platelet-derived Growth Factor and Lysophosphatidic Acid Inhibit Growth Hormone Binding and Signaling via a Protein Kinase C-dependent Pathway*

Liangyou Rui, Stephen F. Archer‡, Lawrence S. Argetsinger, and Christin Carter-Su§

From the Department of Physiology, University of Michigan Medical School, Ann Arbor, Michigan 48109-0622

Growth hormone (GH) regulates body growth and metabolism. GH exerts its biological action by stimulating JAK2, a GH receptor (GHR)-associated tyrosine kinase. Activated JAK2 phosphorylates itself and GHR, thus initiating multiple signaling pathways. In this work, we demonstrate that platelet-derived growth factor (PDGF) and lysophosphatidic acid (LPA) both down-regulate GH signaling via a protein kinase C (PKC)-dependent pathway. PDGF substantially reduces tyrosyl phosphorylation of JAK2 induced by GH but not interferon-γ or leukemia inhibitory factor. PDGF, but not epidermal growth factor, decreases tyrosyl phosphorylation of GHR (by approximately 90%) and the amount of both total cellular GHR (by approximately 80%) and GHR binding (by approximately 70%). The inhibitory effect of PDGF on GH-induced tyrosyl phosphorylation of JAK2 and GHR is abolished by depletion of 4β-phorbol 12-myristate 13-acetate (PMA)-sensitive PKCs with chronic PMA treatment and is severely inhibited by GF109203X, an inhibitor of PKCs. In contrast, extracellular signal-regulated kinases 1 and 2 and phosphatidylinositol 3-kinase 3-kinase appear not to be involved in this inhibitory effect of PDGF. LPA, a known activator of PKC, also inhibits GH-induced tyrosyl phosphorylation of JAK2 and GHR and reduces the number of GHR. We propose that ligands that activate PKC, including PDGF, LPA, and PMA, down-regulate GH signaling by decreasing the number of cell surface GHR through promoting GHR internalization and degradation and/or cleavage of membrane GHR and release of the extracellular domain of GHR.

Growth hormone (GH)* is a circulating peptide hormone

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§ To whom correspondence should be addressed: Dept. of Physiology, University of Michigan Medical School, Ann Arbor, MI 48109-0622. Tel.: 734-763-2561; Fax: 734-647-9523; E-mail: carterss@umich.edu.

The abbreviations used are: GH, growth hormone; GHR, GH receptor; GHBP, GH-binding protein; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; LPA, lysophosphatidic acid; IFN-γ, interferon-γ; LIF, leukemia inhibitory factor; ERK, extracellular signal-regulated kinase; PI 3-kinase, phosphatidylinositol 3-kinase; JAK2, protein kinase J; PMA, 4β-phorbol 12-myristate 13-acetate; PAGF, polyacrylamide gel electrophoresis; MAP, mitogen-activated protein; MEK, MAP kinase/ERK kinase; BSA, bovine serum albumin; hGH, human growth hormone; TNF-α, tumor necrosis factor-α; TACE, TNF-α-converting enzyme; PLC, phospholipase C; aPY, anti-phosphotyrosine antibody 4G10.
phosphorylated (Ser-473), active Akt (immunoprecipitation and 1:5,000 for immunoblotting. Polyclonal anti-amid, Princeton, NJ) and was used at a dilution of 1:250 for recombinant murine LIF was from R&D Systems. Recombinant murine proteins that activate PMA-sensitive PKCs down-regulate GH signaling at least in part reducing the level of GHR. It is likely that other ligands that activate PMA-sensitive PKCs down-regulate GH signaling in a similar fashion.

EXPERIMENTAL PROCEDURES

Materials—Recombinant hGH was a gift of Lilly. hGH was iodinated by the Reproductive Sciences Training Grant Core Facility at the University of Michigan Medical School to a specific activity of ~2,000 μCi/nmol. Recombinant murine EGF was from Collaborative Biomedical Products. Recombinant human PDGF-BB was from Intergen. Recombinant human PDGF-BB was from Intergen. Recombinant murine IFN-γ, LPA, and PMA were from Sigma. Protein A-agarose was from Repligen. Aprotinin, leupeptin, and Trichloroacetic acid were from Roche Molecular Biochemicals. Enhanced chemiluminescence (ECL) detection system was from Amersham Pharmacia Biotech, Wiesbaden and bisindolylmaleimide I (GF109203X) were from Calbiochem. Anti-JAK2 antiserum (9G10) was purchased from Upstate Biotechnology Inc. and was used at a dilution of 1:500 for immunoprecipitation and 1:15,000 for immunoblotting. Polyclonal anti-phosphorylated (Thr-183 and Tyr-185), active MAP kinase antibody 4G10 (Py) was purchased from Upstate Biotechnology Inc. and was used at a dilution of 1:5,700 for immunoblotting. The stock of 3T3-F442A cells was provided by H. Green (Harvard University, Cambridge, MA).

Immunoprecipitation and Immunoblotting—3T3-F442A fibroblasts were grown on 100-mm tissue culture dishes in Dulbecco’s modified Eagle’s medium supplemented with 8% calf serum, 1 mM t-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B. Cells were deprived of serum overnight in the same growth medium except that 1% bovine serum albumin (BSA) was substituted for the calf serum. The deprived cells were treated for various times with the indicated drugs and/or ligands at 37 °C and then rinsed three times with 10 mM sodium phosphate, pH 7.4, 150 mM NaCl, 1 mM NaVO₄. The cells were solubilized in lysis buffer (50 mM Tris pH 7.5, 1% Triton X-100, 150 mM NaCl, 2 mM EGTA, 1 mM NaVO₄, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin), and centrifuged at 14,000 × g for 10 min at 4 °C. Proteins in the supernatant were quantified using BCA Protein Assay Reagent (Pierce). The supernatant was incubated with the indicated antibody on ice for 2 h. The immune complexes were collected on protein A-agarose (50 μl) during 1 h incubation at 4 °C. The beads were washed 3 times with washing buffer (50 mM Tris, pH 7.5, 0.1% Triton X-100, 150 mM NaCl, 2 mM EGTA) and boiled for 5 min in a mixture (80:20) of lysis buffer and SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer (250 mM Tris-HCl, pH 6.8, 10% SDS, 10% β-mercaptoethanol, 40% glycerol, 0.01% bromophenol blue). The eluted proteins were separated by SDS-PAGE (5–12% gradient gel) followed by immunoblotting with the indicated antibody using the ECL detection system.

To assess the total amount of GHR, 3T3-F442A cells were solubilized in the above lysis buffer supplemented with 1% SDS and boiled for 5 min. The presence of GHR proteins in the cell lysates was determined using BCA Protein Assay Reagent. Proteins (50 μg) in the lysates were boiled for 5 min in a mixture (80:20) of lysisates and SDS-PAGE sample buffer, separated by SDS-PAGE, and subjected to immunoblotting using αGHBP. 

125I-hGH Binding Assay—Confluent 3T3-F442A fibroblasts in 6-well plates were deprived of serum overnight and stimulated with the indicated ligands for 45 min. The cells were washed with Krebs-Ringers phosphate (KRP) buffer (128 mM NaCl, 6.7 mM KCl, 1 mM CaCl₂, 2.6 mM MgSO₄, and 10 mM Na₂HPO₄, pH 7.4) containing 1% BSA and then incubated in KRP, 1% BSA containing 125I-hGH (80,000 cpm/well) overnight at 4 °C. The cells were then washed with cold KRP and solubilized in 1 ml of 1 M NaOH for counting the radiation. The concentration of protein in the cell lysates was determined using BCA Protein Assay Reagent. 

RESULTS

PDGF Rapidly Inhibits GH-induced Tyrosyl Phosphorylation of JAK2 and GHR and Reduces the Amount of GHR—To examine whether PDGF cross-talks with GH, 3T3-F442A cells, which express endogenous receptors for PDGF (51, 52) and GH (53), were deprived of serum overnight, preincubated with 25 ng/ml PDGF for 20 min, and stimulated with 50 ng/ml GH for an additional 10 min. JAK2 was immunoprecipitated with αJAK2 and immunoblotted with αPY. The level of tyrosyl phosphorylation of JAK2 was used to assess the extent of activation of JAK2 by GH (6, 16). PDGF substantially inhibits GH-induced tyrosyl phosphorylation of JAK2 (Fig. 1A, upper panel). Densitometric analysis revealed that PDGF reduced GH-induced tyrosyl phosphorylation of JAK2 by approximately 90%. PDGF does not significantly change the amount of JAK2 (Fig. 1A, lower panel). Because GHR is a physiological substrate of JAK2, we tested whether PDGF alters tyrosyl phosphorylation of GHR in response to GH. 3T3-F442A cells were deprived of serum overnight and preincubated with PDGF for 20 min prior to stimulation with 50 ng/ml GH for 10 min. GHR was immunoprecipitated with αGHBP that recognizes the extracellular domain of GHR and immunoblotted with αPY or αGHBP. PDGF dramatically inhibits GH-induced tyrosyl phosphorylation of GHR (Fig. 1B, upper panel). Densitometric analysis of Fig. 1B indicates that PDGF inhibited tyrosyl phosphorylation of GHR by approximately 90%. PDGF by itself does not stimulate tyrosyl phosphorylation of GHR (Fig. 1B, lane 3, upper panel). Interestingly, PDGF appears to decrease total cellular GHR (Fig. 1B, lower panel), compare lanes 1 and 3, 2 and 4). GH stimulates an upward shift in mobility of GHR (Fig. 1B, lanes 1 and 2, lower panel). We believe the mobility shift is caused by phosphorylation of GHR, presumably on tyrosines (6, 54, 55).

Because JAK2 is also activated by interferon-γ (IFN-γ) and leukemia inhibitory factor (LIF) in 3T3-F442A cells (56), we examined whether PDGF inhibits signaling by those ligands. 3T3-F442A cells were deprived of serum overnight and preincubated with 25 ng/ml PDGF for 20 min prior to treatment for 10 min with 10 ng/ml IFN-γ or 25 ng/ml LIF. JAK2 was immunoprecipitated with αJAK2 and immunoblotted with αPY (Fig. 1C and D, upper panel) or αJAK2 (Fig. 1C, C and D, lower panel). IFN-γ (Fig. 1C, lane 2) and LIF (Fig. 1D, lane 2) stimulate tyrosyl phosphorylation of JAK2, as reported previously (56). Surprisingly, PDGF does not inhibit tyrosyl phosphorylation of JAK2 induced by either IFN-γ (Fig. 1C) or LIF (Fig. 1D). These data suggest that PDGF does not directly inhibit JAK2 but rather inhibits specifically GH signaling by a mechanism involving GHR activation and/or the coupling of GHR to JAK2. To address further the specificity of this inhibition of GH signaling by PDGF, we examined whether EGF inhibits GH signaling. The receptors for both PDGF and EGF are receptor tyrosine kinases. 3T3-F442A cells, which express endogenous EGF receptors (51), were deprived of serum overnight and incubated with 125 ng/ml EGF for 20 min prior to 50 ng/ml GH for 10 min. Proteins in cell lysates were immunoprecipitated with αJAK2 (Fig. 1E, lanes 1–4) or αGHBP (lanes 5–8) and immunoblotted with αPY (Fig. 1E, upper panel), αJAK2 (Fig. 1E, lower panel).
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Fig. 1. PDGF, but not EGF, specifically inhibits GH-induced tyrosyl phosphorylation of JAK2 and GHR and reduces the amount of GHR. A and B, 3T3-F442A cells were pretreated with or without PDGF (25 ng/ml) for 20 min prior to GH (50 ng/ml) stimulation for 10 min. Proteins in cell lysates were immunoprecipitated (IP) with αJAK2 (A, lower panel) or αGHBP (B, lower panel). C and D, 3T3-F442A cells were pretreated with or without PDGF (25 ng/ml) for 20 min and then stimulated for 10 min with IFN-γ (10 ng/ml) (C) or LIF (25 ng/ml) (D). JAK2 was immunoprecipitated with αJAK2 and immunoblotted with αPY (upper panel) and reprobed with αJAK2 (lower panel). E, 3T3-F442A cells were pretreated with PDGF (25 ng/ml) or EGF (125 ng/ml) for 20 min prior to GH (50 ng/ml) stimulation for 10 min. Proteins in cell lysates were immunoprecipitated with αJAK2 (lanes 1–4) or αGHBP (lanes 5–8) and immunoblotted with αPY (upper panel). The same blots were reprobed with αJAK2 (lanes 1–4, lower panel) or αGHBP (lanes 5–8, lower panel). IFN-γ, lane 3, upper panel), or αGHBP (Fig. 1E, lanes 5–8, lower panel). Consistent with the data in Fig. 1A, PDGF dramatically reduces GH-induced tyrosyl phosphorylation of JAK2 (Fig. 1E, lane 3, upper panel) and GHR (Fig. 1E, lane 7, upper panel). PDGF reduced the amount of GHR by approximately 80% (Fig. 1E, lower panel). In contrast, EGF neither inhibits GH-induced tyrosyl phosphorylation of JAK2 (Fig. 1E, lane 4, upper panel) and GHR (Fig. 1E, lane 8, upper panel) nor reduces the amount of GHR (Fig. 1E, lane 8 versus 6, lower panel). These results suggest that signaling events that cause down-regulation of GH signaling by PDGF are not shared by EGF.

PDGF-induced inhibition of GH signaling is very rapid. Pretreatment with PDGF for 2 min results in significant inhibition of GH-dependent tyrosyl phosphorylation of JAK2 (Fig. 2A, lane 3) and GHR (Fig. 2B, lane 3). The inhibition of tyrosyl phosphorylation of GHR and JAK2 reaches a maximal level within 15 min pretreatment with PDGF (Fig. 2, A and B). This rapid onset makes it unlikely that PDGF-induced inhibition of GH signaling involves new gene expression or synthesis of new proteins. PDGF-induced inhibition of GH-dependent tyrosyl phosphorylation of JAK2 (Fig. 2C) and GHR (Fig. 2D) is dependent on the dose of PDGF. It is detectable at 1 ng/ml and reaches a maximum with 25 ng/ml PDGF (Fig. 2, C and D).

PDGF also reduces the level of GHR in a time- and dose-dependent manner. The reduction is substantial at 2 min, and maximal within 15 min stimulation with PDGF (Fig. 2B, lower panel). The reduction of GHR is detected at a concentration as low as 1 ng/ml PDGF (Fig. 2D, lane 3, lower panel). Thus, the PDGF-induced inhibition of GH-stimulated tyrosyl phosphorylation of JAK2 (Fig. 2A, A and C, upper panel) and GHR (Fig. 2B, B and D, upper panel) correlates in magnitude, time, and dose with the PDGF-induced reduction of GHR (Fig. 2, B and D, lower panel). This correlation suggests that PDGF inhibits GH signaling by reducing the amount of GHR present in cells.

PDGF decreases the number of GHR on the cell surface available to bind to GH—Because PDGF does not interfere with the activation of JAK2 by IFN-γ (Fig. 1C) or LIF (Fig. 1D), and PDGF-induced inhibition of tyrosyl phosphorylation of JAK2 and GHR correlates with the reduction in the level of cellular GHR (Fig. 2), we reasoned that PDGF does not directly inhibit JAK2 but rather reduces the number of GHR on the cell surface available to bind GH. To examine whether PDGF decreases the number of GHR on the cell surface available to bind GH, 3T3-F442A cells were deprived of serum overnight, treated for 45 min with 25 ng/ml PDGF, 125 ng/ml EGF, or 200 nM PMA, and subjected to a GH binding assay at 4 °C as described under “Experimental Procedures.” PMA has been shown to reduce GH binding in 3T3-F442A cells (57) and in IM9 cells (58) and was used as a positive control. PDGF reduced GH binding by approximately 70% (Fig. 3). PMA decreased GH binding to a slightly greater extent (by approximately 75%). In contrast, EGF did not affect GH binding.

The PDGF-induced Reduction of GHR Requires Neither the ERK Cascade nor PI 3-Kinase—To verify the reduction of total cellular GHR by PDGF described in Figs. 1 and 2, 3T3-F442A cells were deprived of serum overnight and treated with 25 ng/ml PDGF or 125 ng/ml EGF for 40 min. Cells were lysed in lysis buffer containing 1% SDS and boiled for 5 min. No residual cell pellet was observed following this treatment. The whole cell lysates were subjected to SDS-PAGE. Proteins in the cell lysates were immunoblotted with αGHB (Fig. 4A, lanes 2–4). To verify the migration of GHR, αGHB immunoprecipitate was also analyzed on the same SDS-PAGE gel (Fig. 4A, lane 1). PDGF reduced the amount of total cellular GHR by approximately 75% (Fig. 4A, lanes 3 versus 2). In contrast, EGF does not affect the level of GHR (Fig. 4A, lane 4).

PDGF activates multiple signaling molecules and pathways, including the MEK/ERK cascade, the PI 3-kinase/Akt pathway, and the PLC-γ/PKC pathway. To determine which molecules and pathways are involved in PDGF-induced inhibition of GH signaling, 3T3-F442A cells were treated with 25 ng/ml PDGF or 125 ng/ml EGF for 40 min, and proteins in cell lysates were immunoblotted with αPY (Fig. 4B, top panel) antibody to the activated form of mitogen-activated protein kinase which recognizes the activated, dually phosphorylated form of ERKs 1 and 2 (Fig. 4B, middle panel) or antibody to the activated form of Akt phosphorylated on Ser-473 which is the site phosphorylated by PI 3-kinase (Fig. 4B, bottom panel). PDGF stimulates

2 Proteins in the tight band migrating with apparent Mr ~90,000 in Fig. 2, B and D, lower panel, are believed not to be functional GHR, because they are not tyrosyl-phosphorylated in response to GH (Fig. 2, B and D, upper panel).
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**Fig. 2.** PDGF rapidly inhibits GH-induced tyrosyl phosphorylation of JAK2 and GHR and reduces the amount of GHR in a dose-dependent manner. A and B, 3T3-F442A cells were pretreated with PDGF (25 ng/ml) for the indicated times prior to GH (50 ng/ml) stimulation for 10 min. Proteins in cell lysates were immunoprecipitated (IP) with αJAK2 (A) or αGHB (B) and then immunoblotted (IB) with αPY (A and B, upper panel). The blots were reprobed with αJAK2 (A, lower panel) or αGHB (B, lower panel). C and D, 3T3-F442A cells were pretreated for 20 min with the indicated concentrations of PDGF prior to GH (50 ng/ml) stimulation for 10 min. Proteins in cell lysates were immunoprecipitated with αJAK2 (C) or αGHB (D) and then immunoblotted with αPY (C and D, upper panel). The blots were reprobed with αJAK2 (C, lower panel) or αGHB (D, lower panel).

**Fig. 3.** PDGF, but not EGF, reduces GH binding. 3T3-F442A cells were pretreated for 45 min with or without PDGF (25 ng/ml) or PMA (200 nM) and then subjected to a GH binding assay as described under "Experimental Procedures" (n = 3, ± S.E.).

Tyrosyl phosphorylation of two proteins migrating with apparent molecular weights of approximately 175,000 and 145,000 (Fig. 4B, lane 2, top panel), sizes appropriate for the PDGF receptor and PLCγ, respectively. In contrast, EGF stimulates tyrosyl phosphorylation of a protein migrating with apparent molecular weight 160,000, a size appropriate for the EGF receptor (Fig. 4B, lane 3, top panel). PDGF activates both ERKs 1 and 2 (Fig. 4B, lane 2, middle panel) and Akt (Fig. 4B, lane 2, bottom panel). EGF activates ERKs 1 and 2 to an extent similar to that observed with PDGF (Fig. 4B, lane 3, middle panel) but does not activate Akt (Fig. 4B, lane 3, bottom panel). Because PDGF but not EGF reduces the amount of GHR and inhibits GH-induced tyrosyl phosphorylation of JAK2 and GHR, it seems unlikely that ERKs 1 and 2 are involved in the inhibition of GH signaling by PDGF.

Because PDGF appears to be a more potent activator of Akt than EGF (Fig. 4B, bottom panel), a downstream effector of PI 3-kinase (59), we tested whether PI 3-kinase plays a role in the inhibition of GH signaling by PDGF. 3T3-F442A cells were pretreated for 20 min with 200 nM wortmannin, a potent inhibitor of PI 3-kinase, incubated with 25 ng/ml PDGF for 20 min, and then stimulated for 10 min with 50 ng/ml GH. Proteins in cell lysates were immunoprecipitated with αJAK2 (Fig. 6A) or αGHB (Fig. 6B) and immu...
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FIG. 4. ERKs 1 and 2 and PI 3-kinase are not involved in PDGF-induced reduction of the amount of GHR and tyrosyl phosphorylation of JAK2 and GHR. A, 3T3-F442A cells were treated with PDGF (25 ng/ml) or EGF (125 ng/ml) for 40 min and solubilized in lysis buffer containing 1% SDS as described under “Experimental Procedures.” GHR in cell lysates was immunoprecipitated (IP) and immunoblotted (IB) with αGHB P (lane 1). Proteins (50 μg) in the whole cell lysates (lanes 2–4) were immunoblotted with αGHB P. B, 3T3-F442A cells were pretreated with PDGF (25 ng/ml) or EGF (125 ng/ml) for 40 min. Proteins (50 μg) in cell lysates were immunoblotted with αPY (top panel), anti-active MAP kinase (active MAP kinase) (middle panel), and antibody recognizing specifically phosphorylated, active Akt (αAkt) (bottom panel). The migration of molecular weight standards (× 10^5), receptors for EGF (EGFR) and PDGF (PDGFR), PLCγ, ERKs 1 and 2, and activated Akt are indicated. C–E, 3T3-F442A cells were pretreated with or without Wortmannin (Wort) (500 nM) for 25 min and then treated with PDGF (25 ng/ml) for 20 min. The cells were then stimulated with GH (50 ng/ml) for 10 min. Proteins in cell lysates were immunoprecipitated with αJAK2 (C) or αGHB P (D) and immunoblotted with αPY (C and D, upper panel). The same blots were reprobed with αJAK2 (lower panel) and αGHB P (D, lower panel), respectively. Proteins (50 μg) in cell lysates were immunoblotted with αAkt (E).

FIG. 5. Depletion of PKCs blocks the inhibition of GH-induced tyrosyl phosphorylation of JAK2 and GHR by PDGF and PMA. 3T3-F442A cells were pretreated with (A and B, lanes 5–8) or without (A and B, lanes 1–4) PMA (200 nM) for 25 h. Cells were then preincubated with or without PDGF (25 ng/ml) or PMA (200 nM) for 20 min prior to GH (50 ng/ml) stimulation for 10 min. A, JAK2 was immunoprecipitated (IP) with αJAK2 and immunoblotted (IB) with αPY (upper panel). The same blot was reprobed with αJAK2 (lower panel). B, GHR was immunoprecipitated with αGHB P and immunoblotted with αPY (upper panel). LPA also significantly reduces the amount of total cellular GHR (Fig. 7C, lower panel) and GH binding (data not shown). Depletion of PMA-sensitive PKCs blocks the inhibitory effect of LPA on GH-stimulated tyrosyl phosphorylation of JAK2 and GHR (data not shown).

DISCUSSION

We report in this work that PDGF and LPA are potent inhibitors of GH signaling. Pretreating cells with PDGF or LPA dramatically inhibits GH-dependent tyrosyl phosphorylation of the tyrosine kinase JAK2, a key enzyme in GH signaling. Because tyrosyl phosphorylation of JAK2 correlates with its activation (6), PDGF and LPA most likely strongly inhibit GH-dependent activation of JAK2. Consistent with this idea, GH-induced tyrosyl phosphorylation of GHR, a physiological substrate of JAK2, is also severely inhibited by PDGF and LPA. Because activation of JAK2 and tyrosyl phosphorylation of JAK2 and GHR are early obligatory steps in GH signaling (7, 62), it is likely that most, if not all, downstream signaling events are inhibited by PDGF and LPA.

Another important finding of this work is that PDGF and LPA decrease both GH binding and total cellular GHR. PDGF substantially reduces both GH binding and total cellular GHR. The decrease in GH binding and number of GHR roughly correlates with the inhibition of GH-induced tyrosyl phosphorylation of JAK2 and GHR, suggesting that the reduction of GHR is the primary cause of down-regulation of GH signaling by PDGF and LPA. In agreement with this idea, PDGF does not inhibit the activation of JAK2 by LIF or IFN-γ, suggesting that...
PDGF and LPA do not directly inhibit JAK2.

When PMA-sensitive isoforms of PKC are depleted by preincubating cells for 25 h with PMA, neither PDGF nor LPA inhibits GH-induced tyrosyl phosphorylation of JAK2 and GHR, indicating that PMA-sensitive PKCs are required for PDGF- and LPA-induced inhibition of GH action. In support of this hypothesis, GF109203X, a potent inhibitor of PKCs, substantially blocks the inhibition of PDGF-induced tyrosyl phosphorylation of JAK2 and GHR and the reduction in GHR by PDGF. In agreement with an essential role of PKC in down-regulation of GH signaling by PDGF and LPA, activation of PKC by PMA is sufficient to inhibit GH-induced tyrosyl phosphorylation of JAK2 and GHR and reduce the number of GHR. Interestingly, depletion of PMA-sensitive PKCs blocks the ability of both PDGF and PMA to decrease GH signaling, whereas GF109203X, which is capable of almost completely blocking the ability of PMA to inhibit GH signaling, only partially blocks the inhibitory effect of PDGF on GH signaling. One explanation for this apparent discrepancy is that PDGF stimulates a subset of PKCs that are less sensitive to GF109203X (63, 64).

Although it has been reported previously that PMA can regulate levels of GH binding (57, 58, 60), this is the first report of physiological ligands that down-regulate GH binding and GH signaling by a PKC-dependent mechanism. Two models have been proposed to explain the inhibition of GH signaling by PMA-sensitive PKCs (57, 58, 60). In the first model (57, 58), activation of PKCs by PMA leads to a redistribution of GHR within the cell, resulting in a reduction of cell surface GHR and an increase in cytoplasmic GHR. Our data do not support the second half of this model (i.e. cytoplasmic GHR are increased in number). However, we cannot exclude the possibility that PMA activation of PKCs increases the rate of internalization of GHR which are then rapidly degraded, resulting in a decrease in the overall number of GHR.

In the second model (60), activation of PKCs by PMA leads to the activation of a protease that cleaves cell surface GHR. This results in the formation of GHR lacking its extracellular domain and release of GH-binding protein (GHBP, the extracellular domain of GHR) from the cell (shedding). Recent evidence suggests that the PKC-regulated protease may be tumor necrosis factor (TNF)-α-converting enzyme (TACE) or a TACE-like metalloprotease (65). Consistent with a TACE-like metalloprotease cleaving GH and producing GHBP, PMA activation of TACE family members has been shown to lead to ectodomain cleavage of the receptors for TNF-α and transforming growth factor (TGF)-β.
factor-α, the adhesion protein L-selectin, and β-amylloid protein precursor (66, 67). Also consistent with this second model is the finding that the PMA-induced decrease in GHR binding is dependent upon a region of GHR composed of the extracellular and transmembrane domains (57). The best evidence for the production of GHB as a by-product of GHR cleavage comes from studies with GHR from humans and rabbits (69, 63–78), species in which proteolysis is thought to be the major source of GHB (71). However, a preliminary report indicates that formation of GHB as a result of proteolytic cleavage of GHR also occurs with GHR from mice (65), a species for which GHB is also synthesized from the gene for GHR as an alternative splice product (71). If proteolysis accounts for at least some of the PKC-induced decrease in GHB binding, then it seems likely that PDGF, LPA, or any other ligand that activates PMA-sensitive PKCs will stimulate the production of GHB in cells that express GHR. Up to 50% of human serum GHB is believed to bind to GHB (72). The interaction of GH with GHB is proposed to increase the stability of GH. GHB has also been reported to have inhibitory (73) as well as potentiating roles in GH signaling (74–76). Thus, in addition to regulating the ability of an individual cell to respond to GH, ligands such as PDGF and LPA could also increase the local production of GHB and thereby modulate GH signaling in neighboring cells. In addition, because GHB is known to activate members of the TACE family of proteases, it seems likely that PDGF and LPA might stimulate the cleavage of the receptors for TNF-α and transforming growth factor-β, the adhesion protein L-selectin, and/or the β-amylloid protein precursor.

The finding that PDGF and LPA inhibit GH signaling via a PKC-dependent pathway may have important therapeutic implications. Receptors for PDGF and LPA are members of the receptor tyrosine kinase family and the G protein-coupled receptor family, respectively. Many ligands, including numerous chemical compounds and a variety of hormones, cytokines, and growth factors, are able to activate PKCs. It seems likely that any ligand that activates PMA-sensitive PKCs will downregulate GH action. Receptors for a variety of hormones, growth factors, and cytokines that activate PKC, including LPA and PDGF, are expressed in GH target tissues and cells. It is likely that these ligands constantly modulate GH action in vivo by regulating the abundance of GHB on the plasma membrane through a PKC-dependent pathway. Therefore, it is extremely important during GH replacement therapy to be cognizant of the fact that agents that stimulate PKCs will render a given dose of GH much less effective. Similarly, it is important to be cognizant that continuous treatment with agents that stimulate PKCs may inhibit growth.

In summary, we have shown that PDGF and LPA inhibit GH-induced tyrosyl phosphorylation of JAK2 and GHR. PDGF and LPA appear to down-regulate GH signaling by decreasing the number of GHR. PMA-sensitive PKCs, but not ERKs 1 and 2 nor PI 3-kinase, are required for these actions of PDGF and LPA. We propose that any ligand that activates PMA-sensitive isoforms of PKC inhibits GH signaling in a similar fashion.

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