Up-regulation of Endothelial Nitric-oxide Synthase Promoter by the Phosphatidylinositol 3-Kinase γ/Janus Kinase 2/MEK-1-dependent Pathway*

Katarzyna Cieslik‡, Charles S. Abrams§, and Kenneth K. Wu¶

Received for publication, June 19, 2000, and in revised form, October 18, 2000
Published, JBC Papers in Press, October 19, 2000, DOI 10.1074/jbc.M005305200

Our recent study indicates that lysophosphatidylcholine (LPC) enhances Sp1 binding and Sp1-dependent endothelial nitric oxide synthase (eNOS) promoter activity via the mitogen-activated protein kinase/extracellular signal-regulated kinase 1 (MEK-1) signaling pathway (Cieslik, K., Lee, C.-M., Tang, J.-L., and Wu, K. K. (1999) J. Biol. Chem. 274, 34669–34675). To identify upstream signaling molecules, we transfected human endothelial cells with dominant negative and active mutants of Ras and evaluated their effects on eNOS promoter activity. Neither mutant altered the basal or LPC-induced eNOS promoter activity. By contrast, a dominant negative mutant of phosphatidylinositol 3-kinase γ (PI-3Kγ) blocked the promoter activity induced by LPC. Wortmannin and LY 294002 had a similar effect. AG-490, a selective inhibitor of Janus kinase 2 (Jak2), also reduced the LPC-induced Sp1 binding and eNOS promoter activity to the basal level. LPC induced Jak2 phosphorylation, which was abolished by LY 294002 and the dominant negative mutant of PI-3Kγ. LY 294002 and AG-490 abrogated MEK-1 phosphorylation induced by LPC but had no effect on Raf-1. These results indicate that PI-3Kγ and Jak2 are essential for LPC-induced eNOS promoter activity. This signaling pathway was sensitive to pertussis toxin, suggesting the involvement of a G protein in PI-3Kγ activation. These results indicate that LPC enhances Sp1-dependent eNOS promoter activity by a pertussis toxin-sensitive, Ras-independent novel pathway, PI-3Kγ/Jak2/MEK-1/ERK1/2.

Endothelial nitric oxide synthase (eNOS), a member of the NOS family, catalyzes the synthesis in blood vessels of nitric oxide, which plays a key role in maintaining blood pressure homeostasis and vascular integrity (1, 2). eNOS is constitutively expressed primarily in endothelial cells, and its level of expression has been shown to be up-regulated by exercise (3), shear stress (4), hypoxia (5), and lysophosphatidylcholine (LPC; Ref. 6). That LPC is capable of up-regulating the expression of vasoprotective eNOS is intriguing, because LPC has emerged as an important mediator of vascular injury, inflammation, and atherosclerosis (7). It has been postulated that LPC-induced eNOS expression represents a crucial mechanism by which arteries exert their plastic defense against vessel wall injury (8).

LPC is generated from oxidized low density lipoprotein (9) or from inflammatory cells as a result of phospholipase A2 action (10). It possesses a variety of proinflammatory and proatherogenic properties: 1) it increases chemotactic activities of monocytes and T-lymphocytes (11, 12); 2) it has a mitogenic effect on macrophages (13); 3) it activates expression of vascular adhesion molecule-1, intercellular adhesion molecule-1, platelet-derived growth factor, heparin binding epidermal growth factor, and cyclooxygenase-2 (14–17); and 4) it increases thrombomodulin expression and reduces tissue factor and tissue factor pathway inhibitor (18–20). The mechanisms by which LPC exerts the myriad cellular and molecular actions are not entirely clear. It has been suggested that LPC acts as a second messenger to activate signaling molecules such as cAMP, mitogen-activated protein kinase, protein kinase C (PKC), and phosphatidylinositol 3-kinase (PI-3K; Refs. 13, 21–23). LPC has been shown to induce the DNA binding activity of activated protein 1 (24), cAMP response element-binding protein (25), and nuclear factor κB (26). Despite these reports, the signaling pathways that lead to transcriptional up-regulation of these proinflammatory and growth factor genes remain unclear. By contrast, the transcriptional regulation of eNOS is more extensively investigated. The basal eNOS promoter activity depends on binding of Sp1 to an Sp1 cognate site (−90 to −104) on the human eNOS 5′-flanking promoter region (27, 28). In our previous study, we have shown that LPC up-regulates eNOS promoter activity by augmenting specifically the Sp1 binding activity (29). We have further shown that LPC selectively activates extracellular signal-regulated kinase 1/2 (ERK1/2) via mitogen-activated protein kinase/extracellular signal-regulated kinase 1 (MEK-1), and PD98059 (2′-amino-3′-methoxyflavone), a selective inhibitor of MEK-1, abrogated the Sp1-dependent eNOS promoter up-regulation, consistent with an essential role of MEK-1 and ERK1/2 in LPC-induced promoter activity (30). The signaling pathway upstream of MEK-1 has not been reported. In this study, we searched for upstream signaling kinases by using dominant negative mutants or selective pharmacological inhibitors, or both. Here, we report identification of PI-3Kγ as an essential signaling molecule in MEK-1 activation with subsequent Sp1-dependent eNOS promoter up-regulation by LPC. Our results...
further show that PI-3Kα activates a downstream Janus kinase 2 (Jak2), which in turn activates MEK-1. Neither Ras nor Raf-1 activation is required for eNOS promoter activation by LPC.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Two human endothelial cell lines, ECV304 and EAhy926 cells, were used in our experiments, which yielded similar results. ECV304 cells were cultured in Medium 199 (Life Technologies, Inc.) containing 10% fetal bovine serum (FBS). EAhy926 cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies) with 10% fetal bovine serum, containing 10% FBS and HAT supplement (10 μM hypoxanthine and 1.6 μM thymidine) (Life Technologies). Unless otherwise indicated, both types of cells were incubated in medium containing 0.5% FBS for 16 h before experiments.

**Construction of Expression Vectors**—A 5'-flanking fragment of the eNOS promoter at nucleotide positions from −1322 to +22 was obtained by polymerase chain reaction, using genomic DNA as a template and synthetic oligomers as primers: EN1322G (5'-GGGAAGCTTGTGACTGTGCCGTCCTCTTG-3') and synthetic oligomers as primers: EN1322G (5'-GGGAAGCTTGTGACTGTGCCGTCCTCTTG-3'). The polymerase chain reaction product purified from an agarose gel was digested with BglII-HindIII and cloned into a promoterless luciferase expression vector, pGL3.

**PI-3K Dominant Negative Mutant Constructs**—Two dominant negative constructs with high glucose and l-glutamine, containing a constitutively active Ras mutant, a constitutively active Ras mutant, or an empty vector, pCMV5, as described previously (31, 32). The Δp85 construct lacks amino acid residues 478–513 of the p85 subunit and functions as a dominant negative mutant for PI-3Kα, β, and δ. The Δ110γ construct lacks amino acids 948–981 and functions as a dominant negative mutant of PI-3Kγ. Dominant negative Ras (S17N) and dominant active Ras (Q61L) mutants and the control vector pUSEamp (+) were obtained from Upstate Biotechnology.

**Transfection Experiments**—To evaluate the effects of mutant constructs on eNOS promoter activity, cells grown in 35-mm wells at ~50% confluence were incubated with 2 μg eNOS promoter construct at 37 °C for 30 min followed by addition of 2 μg Ras or PI-3K mutant constructs with 10 μl Lipofectin for 5 h. Medium was removed and replaced with complete medium for 24 h. Cells were washed, incubated in medium containing 0.5% FBS for 16 h, and then incubated in fresh medium containing 5% FBS in the presence or absence of 100 μM LPC for 5 h. Cells were harvested, and the promoter activity was measured. For Sp1 binding experiments, cells grown in 100-mm dishes to ~50% confluence were treated with 16 μg of mutant constructs and 80 μl Lipofectin for 5 h. Cells were washed and incubated in complete medium for 24 h, and then medium containing 0.5% FBS for 15 h. Cells were again washed and incubated in fresh medium containing 5% FBS with or without 100 μM LPC for 3 h. Cells were harvested, nuclear extracts were prepared, and the gel shift assay was performed as described below.

**eNOS Promoter Activity**—eNOS promoter activity was determined by a procedure described previously (28). In brief, cells were incubated in serum-free medium containing a mixture of 10 μl Lipofectin and 2 μg eNOS promoter-luciferase construct at 37 °C for 5 h. Medium was removed, and cells were washed and incubated with fresh complete medium for 24 h. The medium was removed and replaced with fresh medium containing 0.5% FBS. Cells were washed and incubated in fresh medium containing 5% FBS with or without the pharmacological inhibitor to be investigated for 1 h before addition of 100 μl LPC for 5 h at 37 °C. For the pertussis toxin (PTX) experiment, the medium was removed and replaced with fresh medium containing 0.5% FBS with or without PTX for 16 h. The medium was replaced with fresh medium containing 5% FBS to which 100 μl LPC was added. The cells were incubated for an additional 5 h at 37 °C. The cells were harvested, and the expressed luciferase activity was measured in a luminometer (BioSkan). The results were expressed as a relative light units/mg protein.

**Genistein, wortmannin, LY 294002** (2-(4-morpholinyl)-8-phenyl-4-1H-naphthalen-1-yl-1,4-benzopyran-4-one), One 490 (N-cyano-(3,4-dihydroxy)-N-[(3-benzyl)-5-benzoylamin]- and PTX were purchased from Calbiochem, and LPC was purchased from Avanti.

**Electrophoretic Mobility Shift Assay**—The gel shift assay was performed as described previously (29). Endogenous peroxisome proliferator-activated receptor γ co-activator 1 was removed from fresh medium containing 5% FBS in the presence or absence of a pharmacological inhibitor followed by addition of LPC for 3 h. 10 μg of nuclear extracts from these treated cells were incubated in a binding buffer containing 25 μM Tris-HCl, pH 8.0, 50 mM KCl, 10% glycerol, 50 μM bovine serum albumin, 6.0 μM MgCl2, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 1 μM ZnCl2, 1.5 μg poly(dI-dC), and 0.05% Nonidet P-40 at room temperature for 15 min. A [γ32P]ATP-labeled Sp1 oligonucleotide probe (29) was added and incubated at room temperature for 15 min. The mixture was electrophoresed at 12.5 V/cm on a 5% polyacrylamide gel with a buffer containing 0.5× Tris borate-EDTA and 0.5% Nonidet P-40. The gel was vacuum dried and autoradiographed. We have previously demonstrated that the gels were prepared from EV304 or EAhy926 cells form two retarded bands with a labeled probe containing the canonical Sp1 motif (29). These two bands were competed out by a 100-fold molar excess of unlabeled probe but not by Sp1-mutated probe. Our previous experiments also demonstrated that LPC treatment invariably enhanced Sp1 binding by ~2-fold (29), and the augmented Sp1 binding was responsible for the eNOS promoter activity. These results have been highly reproducible. Only the two shifted bands of Sp1-DNA complexes are shown in the figures. The denser band was subject to densitometry in all experiments.

**Jak2 Assay**—The Jak2 assay was assayed by its autophosphorylation (33). Cells were incubated in fresh medium containing 5% FBS in the presence or absence of an inhibitor for 1 h before addition of LPC for 15 min. Cells were then washed twice with ice-cold buffered saline containing 0.5% phenylmethylsulfonyl fluoride, 0.5 mM Na3VO4, and 0.05 mM NaF and then lysed in a Triton X-100 lysis buffer containing 1% Triton X-100, 20 mM Tris-HCl, pH 8.0, 137 mM NaCl, 10% glycerol, 2 mM EDTA, 1.5 mM MgCl2, 1 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride, and 0.05 mM NaF for 1 h at 4 °C with mild agitation. Detergent-solubilized samples were centrifuged at 12,000 rpm. Protein concentration was determined using a bicinchoroneinic acid protein assay (Pierce). 500 μg of the cytosolic proteins were immunoprecipitated with an anti-Jak2 antibody (Santa Cruz) overnight in immunoprecipitation buffer containing PBS, pH 7.4, 0.5 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 1 μg/ml leupeptin. Protein G Plus agarose (Santa Cruz) was added to absorb immune complexes, and after washing twice with immunoprecipitation buffer and once with Jak2 kinase buffer (0.02 mM 1,4-piperazinedithanesulfonic acid, 3 mM MnCl2, and 1 mM/ml leupeptin), the immune complexes were precipitated in 50 μl of Jak2 kinase buffer containing 10 μl [γ32P]ATP for 30 min at 30 °C. The reaction was stopped by addition of 2× Laemmlli buffer. Proteins were boiled for 5 min and subjected to SDS-polyacrylamide gel electrophoresis and autoradiography.

**RESULTS**

**Ras Is Not Required for Basal or LPC-induced eNOS Promoter Activity**—Results from our previous report (30) indicate that LPC selectively activates MEK-1 and ERK1/2 via which Sp1 binding activity was increased and eNOS promoter activity was augmented. To determine whether LPC-induced eNOS expression depends on activation of upstream Ras, we transfected EV304 cells or EAhy926 cells with a Ras dominant negative mutant, a constitutively active Ras mutant, or an empty vector and determined the basal and LPC-induced eNOS promoter activity by cotransfected the cells with the luciferase expression vectors. Basal and LPC-induced eNOS promoter activity (Fig. 1A) in cells transfected with the control vector pUSE were comparable with those in native cells as previously reported (29). Neither the dominant active nor the dominant negative mutants significantly altered the basal or LPC-induced eNOS activities (Fig. 1A). In accord with this, neither mutant had a significant effect on Sp1 binding activity (Fig. 1, B and C). It has been previously reported that transforming growth factor β1 (TGFβ1) activates the Ras—MEK1 signaling pathway in several types of cells including endothelial cells (34–37), which may mediate up-regulation of the promoter function of
diverse genes in various cells (36, 37). We therefore evaluated the effect of the dominant negative mutant of Ras on TGFβ1-induced MEK-1 activation measured as phosphorylated MEK-1 on immunoblots. Our results confirmed that TGFβ1 (5 ng/ml) activated MEK-1, which was abrogated by the dominant negative mutant of Ras but not by the control vector (Fig. 1, D and E). Taken together, these results indicate that the signaling pathway by which LPC induced eNOS promoter activity bypasses Ras as contrasted to the requirement of Ras for the TGFβ1 signaling in endothelial cells.

PI-3Kγ Activation Is Essential for Sp1-dependent eNOS Promoter Function—To search for an upstream kinase that transmits signals to augment the eNOS promoter activity via the MEK-1/ERK1/2 pathway, we evaluated the effects of selective PI-3K inhibitors on eNOS promoter activity and Sp1 binding activity. Neither wortmannin (50 nM) nor LY 294002 (50 μM) inhibited basal Sp1 binding activity (Fig. 2, A and B) or eNOS promoter activity (Fig. 2C) but reduced the LPC-induced Sp1 binding activity (Fig. 2A and B) and eNOS promoter activity (Fig. 2C) to the basal level. Because neither wortmannin nor LY 294002 is a selective inhibitor of PI-3K isoforms, we used Dp110 and Dp85 to identify the isoform of PI-3K that is involved in LPC-induced eNOS transcription. LPC-induced eNOS promoter activity was blocked by Δp110γ and Δp85 to identify the isoform of PI-3K that is involved in LPC-induced eNOS transcription. LPC-induced eNOS promoter activity was blocked by Δp110γ transfection but not by Δp85 transfection (Fig. 3). Neither mutant reduced the basal activity, and the control vector had no effect on LPC-induced or basal promoter activity (Fig. 3). Thus, LPC-induced eNOS promoter function depends on activation of PI-3Kγ. PI-3Kγ activation has been reported to be linked to an upstream Gi activation, when Gipγ is dissociated from Giα and
interacts with PI-3Kγ (38). We therefore determined whether the LPC-induced eNOS promoter function was PTX sensitive. The eNOS promoter activity induced by LPC was abrogated by PTX treatment (Fig. 4A). This was confirmed by a reduction of the Sp1 binding activity to the basal level by PTX (100 ng/ml; Fig. 4, B and C). Neither the basal eNOS promoter activity nor the basal Sp1 binding activity was perturbed by PTX. These results suggest that the action of LPC on eNOS promoter activity is mediated by PTX-sensitive, Gt-coupled activation of PI-3Kγ.

Jak2 Acts Downstream of PI-3Kγ—Genistein at concentrations of ≥50 μM inhibited Sp1 binding activity (Fig. 5, A and B) but had no significant effect at 5 μM. The eNOS promoter activity was also inhibited by genistein at 50 μM (Fig. 5C). Because receptor tyrosine kinase is reported to be inhibited by genistein at a 5 μM concentration (IC₅₀ = 2.6 μM; Ref. 39), whereas cytosolic tyrosine kinase is inhibited by higher genistein concentrations, our results suggest the involvement of a cytosolic tyrosine kinase in eNOS promoter activity. AG-490, a selective inhibitor of a cytosolic tyrosine kinase, Jak2, reduced the Sp1 binding to the basal level (Fig. 6, A and B) with a concordant suppression of eNOS promoter activity. AG-490, a selective inhibitor of a cytosolic tyrosine kinase, Jak2, reduced the Sp1 binding to the basal level (Fig. 6, A and B) with a concordant suppression of eNOS promoter activity. AG-490, a selective inhibitor of a cytosolic tyrosine kinase, Jak2, reduced the Sp1 binding to the basal level (Fig. 6, A and B) with a concordant suppression of eNOS promoter activity.
suggesting that Jak2 activation is downstream of PI-3K activation. This was supported by the results demonstrating that Jak2 phosphorylation was abolished in cells transfected with Δp110 but not with Δp85 (Fig. 7C).

MEK-1 Is the Downstream Target of PI-3K and Jak2—We then determined whether Raf-1 or MEK-1 is the downstream target of Jak2. We measured the levels of phosphorylated MEK-1 and Raf-1-P, because phosphorylation of these two signaling molecules correlate with their activation. Raf-1-P was detected in unstimulated cells, which was not enhanced by LPC (Fig. 8A). AG-490 (50 μM), LY 294002 (50 μM), and PTX (100 ng/ml) had no effect on Raf-1-P levels (Fig. 8A). The Raf-1 protein levels were also unaffected by any of the treatments (data not shown). By contrast, the phosphorylated MEK-1 level was increased by ~2-fold in response to LPC stimulation, and this increase was blocked by AG-490 (50 μM), as well as by LY 294002 (50 μM) and PTX (100 ng/ml; Fig. 8, B and C). The MEK-1 protein level was unaltered (Fig. 8D). These results are consistent with MEK-1 as the target of the PI-3K/Jak2 signaling pathway.

Effect of PTX, AG-490, and LY 294002 on eNOS Protein Levels—The results so far indicate that LPC increased Sp1-mediated eNOS promoter activity via the Gt-coupled PI-3K→Jak2→MEK1 pathway. To confirm that this pathway is involved in eNOS protein expression, we evaluated the effects of several inhibitors on eNOS protein levels. LPC increased eNOS protein over the basal level by ~2-fold, which is in agreement with the extent of increase in eNOS promoter activity. The eNOS protein increase was reduced to the basal level by PTX, AG-490, and LY 294002 (Fig. 9, A and B).

DISCUSSION

Several laboratories including ours have reported that LPC activates MEK-1, which in turn activates ERK1/2 (21, 30). Results from our previous studies further indicate that the MEK-1/ERK1/2 signaling pathway is essential for Sp1-dependent eNOS promoter activity induced by LPC (30). In the present study, we provide information for the first time that activation of the MEK-1 signaling pathway leading to eNOS promoter up-regulation depends on activation of upstream PI-3K and Jak2. Several pieces of evidence from the present study indicate that PI-3Kγ and Jak2 activations are essential for eNOS promoter up-regulation by LPC: 1) wortmannin and LY 294002 block LPC-induced MEK-1 phosphorylation (Fig. 8B), Sp1 binding (Fig. 2, A and B), and eNOS promoter activity (Fig. 2C); 2) the dominant negative mutant of p110γ selectively abrogated eNOS promoter activity increased by LPC (Fig. 3); and 3) AG-490, a selective inhibitor of Jak2, reduced LPC-induced Sp1 binding (Fig. 6, A and B), eNOS promoter activity (Fig. 6C), and MEK-1 phosphorylation (Fig. 8B) to the basal level. Importantly, our results indicate that Jak2 acts downstream of PI-3Kγ. Compelling evidence to support this conclusion includes the abrogation of Jak2 autophosphorylation by Δ110γ (Fig. 7C) as well as by LY 294002 (Fig. 7A). By contrast, the conventional upstream signaling molecules of MEK-1, i.e. Ras and Raf-1, do not play a significant role in signaling LPC-induced eNOS promoter activity. Neither dominant negative nor dominant active Ras mutants exerted any effect on basal or LPC-induced Sp1 binding and eNOS promoter activity (Fig. 1). Furthermore, LPC did not increase Raf-1 phosphorylation (Fig. 8A). Taken together, these findings indicate that LPC induces eNOS promoter function in endothelial cells by a novel signaling pathway involving PI-3Kγ→Jak2→MEK1→ERK1/2.
PI-3K catalyzes the synthesis of phosphatidylinositol 3,4,5-trisphosphate, which is an important second messenger for diverse cellular responses (40). At least four PI-3K isoforms have been characterized (41). Heterodimeric PI-3Kα, PI-3Kβ, and PI-3Kδ constitute a p110 catalytic subunit and a p85 or p55 regulatory subunit. The heterodimeric PI-3Kγ constitutes a p110γ catalytic subunit, which does not interact with p85 adapters but forms a dimer with a p101 regulatory subunit. The dominant negative deletion mutants Δp110γ and Δp85 specifically inhibit PI-3Kγ and PI-3Kα, -β, or -δ, respectively. Our results clearly show that Δp110γ but not Δp85 blocked LPC-induced eNOS promoter activity signaled via the MEK-1/ERK1/2 pathway. Both PI-3Kγ and PI-3Kα have been shown to transmit signals to activate MEK-1 in different cell types via a common pathway involving Shc, Grb2, and SOS (son of sevenless) with subsequent activation of Ras and Raf-1 (42–44). eNOS promoter activation by LPC does not signal through this pathway, because neither Ras nor Raf-1 activation was required for LPC-induced eNOS promoter activity. Alternatively, G_{iβγ}-stimulated PI-3Kγ has recently been reported to activate PKCζ (45, 46), and PKCζ activates MEK-1 independently of Ras and Raf-1 (44). The LPC-induced eNOS promoter up-regulation may be signaled through this pathway. In our preliminary work, we found that several PKC inhibitors blocked ERK1/2 activation and eNOS promoter activity, supporting the involvement of a PKC in this pathway. Furthermore, Jak2

\[ \text{fig. 5. Effect of genistein (GE) on Sp1 binding and eNOS promoter activity.} \]

\[ \text{fig. 6. Suppression of Sp1 binding and eNOS promoter activity by a selective Jak2 inhibitor, AG-490 (AG; 50 μM).} \]

\[ \text{C.-M. Lee, K. Cieslik, and K. K. Wu, unpublished data.} \]
activation in thrombin-stimulated platelets (G protein-coupled receptor) is downstream of PKC activation (47). It is possible that a PKC isoform, such as atypical PKC\(\beta\)\(i\), serves as an intermediate signal between PI-3K\(g\) and Jak2 in the LPC-induced MEK-1 activation. Further studies are required to evaluate this possibility. It was reported that PI-3K\(g\) possesses a protein kinase activity that signals to ERK1/2 in addition to the well-characterized lipid kinase activity, which signals to protein kinase B/Akt (48). It is unclear whether signaling of PI-3K\(g\) to Jak2 and MEK-1 is mediated by its protein kinase activity or via lipid kinase activity, nor is it clear whether the Akt pathway is involved in ERK1/2-mediated promoter up-regulation by LPC. Nevertheless, it should be noted that Akt activation leads to phosphorylation of a serine residue located at the C-terminal region of eNOS, and the phosphorylated eNOS exhibits enhanced catalytic activity (49). Thus, the PI-3K pathway(s) occupies a central position in regulating eNOS activity.

Jak2 is a member of the Janus kinase family of nonreceptor protein tyrosine kinases, which consists of three additional members: Jak1, Jak3, and Tyk2. Each member has a conserved C-terminal kinase domain. Jak2 was reported to activate PI-3K\(a\), but the exact mechanism by which these two molecules interact is unclear (50). This Jak2-dependent PI-3K\(a\) pathway is unlikely to signal LPC-induced eNOS promoter function, because transfection of \(\Delta p85\), which blocks PI-3K\(a\) activity, had no effect on eNOS promoter activity. Jak2 has been reported to be directly associated with and stimulated by the angiotensin II receptor (51, 52). The relevance of these reports to LPC-induced signaling Jak2 is unclear, because it is unknown whether the action of LPC is receptor-mediated. However, on the basis of our experimental results, it is unlikely that Jak2 is directly activated by LPC receptor, even if such a receptor exists. Our results clearly show that Jak2 activation is downstream of PI-3K\(a\). A previous study has shown that PI-3K\(a\) activates Bruton’s tyrosine kinase in lymphocytes (53). Further studies are likely to identify additional nonreceptor protein-tyrosine kinases that are activated by PI-3K\(a\). The mechanism by which PI-3K\(a\) activates Jak2 is unclear. It is possible that phosphatidylinositol 3,4,5-trisphosphate generated by PI-3K\(a\) may bind to the JH motif (Jak homology domain) located in the catalytic domain of Jak2, leading to Jak2 activation. The JH motif is structurally similar to the Src homology 2 domain (SH2), and phosphatidylinositol 3,4,5-trisphosphate is known to bind the Src homology 2 domain for activation of kinases. Alternatively, PI-3K\(a\) may activate Jak2 through its protein kinase activity (48).

It has been reported in HeLa and 3T3 cells that LPC can also activate the c-Jun N-terminal kinase pathway and thereby increase AP1-dependent gene transcription (24). We did not
perturbation. LPC is an amphiphatic molecule and has been shown to transit through plasma membrane and to enter into cells at a rapid rate (59). It is unlikely that it will exert a specific effect on activation of G proteins. Alternatively, its action may be mediated by G_{i}-coupled receptor activation. In view of the important roles that LPC plays in diverse pathophysiological processes, it should be valuable to determine whether its action is mediated by a specific receptor. Identification of such a specific receptor should have important therapeutic implications.

Acknowledgment—We thank Susan Mitterling for editorial assistance.

REFERENCES

1. Moncada, S., Palmer, R. J., and Higgs, E. A. (1991) Pharmacol. Rev. 43, 109–142
2. Wu, K. K. (1995) Adv. Pharmacol. 33, 179–207
3. Sessa, W. C., Pritchard, K., Seyedi, N., Wang, J., and Hintze, T. H. (1994) Circ. Res. 74, 3349–3353
4. Nadeau, S., Philippe, M., Arnal, J. F., Michel, J. B., and Soubrier, F. (1996) Circ. Res. 79, 857–863
5. Arnett, U. A., McMillan, A., Dinerman, J. L., Ballermann, B., and Lowenstein, C. J. (1996) J. Biol. Chem. 271, 15069–15073
6. Zembowicz, A., Tang, J.-L., and Wu, K. K. (1995) J. Biol. Chem. 270, 17006–17010
7. Witztum, J., and Steinberg, D. (1991) J. Clin. Invest. 88, 1785–1792
8. Wu, K. K. (1995) Proc. Natl. Acad. Sci. U. S. A. 110, 163–170
9. Parthasarathy, S., Streinbrecher, U. P., Barnett, J., Witztum, J. L., and Steinberg, D. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 3000–3004
10. Asaoka, Y., Yoshida, K., Sasaki, Y., Nishizuka, Y., Murokami, M., Kudo, I., and Inoue, K. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 716–719
11. Quinn, M. T., Parthasarathy, S., and Steinberg, D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2805–2809
12. Asaoka, Y., Oka, M., Yoshida, K., Sasaki, Y., and Nishizuka, Y. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6447–6451
13. Sakai, M., Miyazaki, A., Hakama, H., Sasaki, T., Yui, S., Yamazaki, M., Shihchi, M., and Horisaki, T. (1994) Hypertension 23, 248–254
14. Kune, N., and Gimbrone, M. A. (1994) J. Clin. Invest. 93, 907–911
15. Kune, N., Cybulski, M. L., and Gimbrone, M. A. (1992) J. Clin. Invest. 90, 1138–1144
16. Nakano, T., Raines, E. W., Abraham, J. A., Klagsbrun, M., and Ross, R. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1069–1073
17. Zembowicz, A., Jones, S. L., and Wu, K. K. (1995) J. Clin. Invest. 96, 1688–1692
18. Yuen, Y., Schoenwaelder, M. S., Salem, H. M., and Jackson, S. P. (1996) J. Biol. Chem. 271, 27090–27098
19. Engel, B., Riessen, S., Brand, K., Page, S., Lentschert, A., Ulmer, A. J., and Gerlach, E. (1999) Atherosclerosis. Thromb. Vasc. Biol. 19, 47–53
20. Sato, N., Kakame, K., Miyata, T., and Kato, H. (1998) Thromb. Haemost. 79, 217–221
21. Wang, J. T., Tran, K., Pierce, G. N., Chan, A. C. O., and Choy, P. C. W. (1998) J. Biol. Chem. 273, 6830–6836
22. Yamakawa, T., Eguchi, S., Yamakawa, Y., Motley, E. D., Namaguchi, K., Minamino, T., Nishimura, Y., and Inoue, S. (1998) Hypertension 23, 248–254
23. Nishio, H., Horiiuchi, H., Ariai, H., and Kita, T. (1998) FEBS Lett. 441, 63–66
24. Fang, X., Gibson, S., Flowers, M., Furui, T., Bast, R. C., and Mills, G. B. (1997) J. Biol. Chem. 272, 13683–13689
25. Ueno, H., Yone, K., Nai, M., Morimoto, M., Kataoka, H., Ochi, H., Nishizuka, K., Miyazaki, A., and Inagami, T. (1998) Hypertension 31, 159–163
26. Zembowicz, A., Cieslik, K., Zembowicz, A., Tang, J.-L., and Wu, K. K. (1995) Biochim. Biophys. Acta 1233, 93–98
27. Zhang, R., Min, W., and Sessa, W. C. (1995) J. Biol. Chem. 270, 15320–15326
28. Tang, J.-L., Zembowicz, A., and Wu, K. K. (1995) Biochim. Biophys. Res. Commun. 213, 673–680
29. Cieslik, K., Zembowicz, A., Tang, J.-L., and Wu, K. K. (1998) J. Biol. Chem. 273, 14885–14890
30. Cieslik, K., Lee, C.-M., Tang, J.-L., and Wu, K. K. (1999) J. Biol. Chem. 274, 34669–34675
31. Ama, and Abrams, C. S. (1999) J. Biol. Chem. 274, 26730–26735
32. Mas, A., Metjian, A., Bagrodia, S., Taylor, S. A., and Abrams, C. S. (1998) Mol. Cell. Biol. 18, 4744–4751
33. Torz, P., Doppier, H., Pfannenmaier, K., and Muller, G. (1999) FEBS Lett. 464, 159–163
34. Santillán, J. F., Igleisas, M., Frontelo, P., Martinez, J., and Quintanilla, M. (2000) Biochem. Biophys. Res. Commun. 273, 521–527
35. Yamamoto, H., Asahara, T., Tanaka, H., Ogawa, W., Abe, M., Akira, T., and Ueno, H. (1999) Eur. J. Biochem. 264, 110–119
36. Hu, P. P., Shen, X., Huang, D., Liu, Y., Counter, C., and Wang, X.-F. (1999) J. Biol. Chem. 274, 35381–35387
37. Inoue, N., Venema, R. C., Sayegh, H. S., Ohara, Y., Murphy, T. J., and Harrison, D. G. (1995) Arterioscler. Thromb. Vasc. Biol. 15, 1255–1261
38. Lopez-Ilasaca, M., Crespo, P., Pellici, G., Gutkind, J. S., and Wetzker, R. (1997) Science 275, 394–397
39. Akita, Y., Tshida, T., Nakagawa, S., Ogawa, W., Watanabe, S., Itoh, N., Shibu, M., and Fukumi, Y. (1987) J. Biol. Chem. 262, 5592–5597
40. Toker, A., and Gantley, L. C. (1997) Nature 387, 673–676
41. Vanhaesebroeck, B., Leefers, S. J., Panayotou, G., and Waterfield, M. D.
42. Leopoldt, D., Hanck, T., Exner, T., Maier, U., Wetzker, R., and Nurnberg, B. (1998) *J. Biol. Chem.* 273, 7024–7029.
43. Van Biesen, T., Hawes, B. E., Luttrell, D. K., Krueger, K. M., Touhara, K., Porfiri, E., Sakaue, M., Luttrell, L. M., and Lefkowitz, R. J. (1995) *Nature* 376, 781–784.
44. Hawes, B. E., Luttrell, L. M., Van Biesen, T., and Lefkowitz, R. J. (1996) *J. Biol. Chem.* 271, 12133–12136.
45. Wang, Y.-X., Dhulipala, P. D. K., Li, L., Benovic, J. L., and Kotlikoff, M. I. (1999) *J. Biol. Chem.* 274, 13859–13864.
46. Takeda, H., Matezaki, T., Takada, T., Naguchi, T., Yamao, T., Tauda, M., Ochi, F., Fukunaga, K., Inagaki, K., and Kasuga, M. (1999) *EMBO J.* 18, 386–395.
47. Rodriguez, B., and Watson, S. P. (1994) *FEBS Lett.* 352, 335–338.
48. Bondeva, T., Pirolo, L., Bulgarelli-Leva, G., Pubio, I., Reinhard, W., and Wymann, M. P. (1998) *Science* 282, 293–296.
49. Fulton, D., Gratton, J. P., McCabe, T. J., Fontana, J., Fujio, Y., Walsh, K., Franke, T. F., Papapetropoulos, A., and Sessa, W. C. (1999) *Nature* 399, 597–601.
50. Al-Shami, A., and Naccache, P. H. (1999) *J. Biol. Chem.* 274, 5333–5338.
51. Marrero, M. B., Schleffer, B., Paxton, W. G., Heerdt, L., Berk, B. C., Delafaceaine, P., and Bernstein, K. E. (1995) *Science* 375, 247–250.
52. Ali, M. S., Sayeski, P. P., Dirksen, L. B., Hayner, D. J., Marrero, M. B., and Bernstein, K. E. (1997) *J. Biol. Chem.* 272, 23382–23388.
53. Li, Z., Wahl, M. I., Equinao, A., Stephens, L. R., Hawkins, P. T., and Witte, D. N. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 13820–13825.
54. Rikitake, Y., Kawashima, S., Yamashita, T., Ueyama, T., Ishido, S., Hotta, H., Hirata, K., and Yokoyama, M. (2000) *Atheroscler. Thromb. Vasc. Biol.* 20, 1006–1012.
55. Sato, N., Okuma, K., Shimokaise, K., Kato, H., and Miyata, T. (1998) *J. Biochem.* (Tokyo) 123, 1119–1126.
56. Shingu, T., and Bornstein, P. (1994) *J. Biol. Chem.* 269, 32551–32557.
57. Neish, A. S., Khachigian, L. M., Park, A., Baichwal, V. R., and Collins, T. (1995) *J. Biol. Chem.* 270, 28903–28909.
58. Chen, Y. Q., Su, M., Walaia, R. R., Hao, Q., Covington, J. W., and Vaughan, D. E. (1998) *J. Biol. Chem.* 273, 8225–8231.
59. Mohandas, N., Wyatt, J., Mel, S. F., Rossi, M. E., and Shohet, S. B. (1982) *J. Biol. Chem.* 257, 6537–6543.
