Protein Kinase Ca-Induced p115RhoGEF Phosphorylation Signals Endothelial Cytoskeletal Rearrangement*

Michael Holinstat‡, Dolly Mehta‡§, Tohru Kozasa‡, Richard D. Minshall‡¶, and Asrar B. Malik‡

From the Departments of §Pharmacology and ¶Anesthesiology, University of Illinois, College of Medicine, Chicago, Illinois 60612

Heterotrimeric G-proteins of the Gα12/13 family activate Rho GTPase through the guanine nucleotide exchange factor p115RhoGEF. Because Rho activation is also dependent on protein kinase Ca (PKCa), we addressed the possibility that PKCa can also induce Rho activation secondary to the phosphorylation of p115RhoGEF. Studies were made using human umbilical vein endothelial cells in which we addressed the mechanisms of PKCa-induced Rho activation and its consequences on actin cytoskeletal changes. We observed that PKCa associated with p115RhoGEF within 1 min of thrombin stimulation and p115RhoGEF phosphorylation was dependent on PKCa. Inhibition of PKCa-dependent p115RhoGEF phosphorylation prevented the thrombin-induced Rho activation, indicating that the response occurred downstream of PKCa phosphorylation of p115RhoGEF. The regulator of G-protein signaling domain of p115RhoGEF, a GTPase activating protein for G12/13, also prevented thrombin-induced Rho activation, indicating the parallel requirement of G12/13 in signaling Rho activation via p115RhoGEF. These data demonstrate a pathway of Rho activation involving PKCa-dependent phosphorylation of p115RhoGEF. Thus, Rho activation in endothelial cells and the subsequent actin cytoskeletal re-arrangement require the cooperative interaction of both G12/13 and PKCa pathways that converge at p115RhoGEF.

Endothelial cells form the primary barrier between blood constituents and the underlying tissues (1, 2). Regulation of endothelial barrier function is required for the maintenance of tissue fluid balance (2). Rho, a monomeric GTPase, plays an important role in the mechanism of loss of endothelial barrier function (2, 3) by inducing the formation of intercellular gaps through actomyosin-driven endothelial cell retraction (4–11). Activation of Rho requires GDP/GTP exchange dependent on guanine nucleotide exchange factors (GEFs). Thus, GEFs are critical regulators of Rho activation, and thereby in signaling Rho-dependent actin cytoskeletal rearrangement and the subsequent loss of endothelial barrier function.

Thrombin has been used to address the signaling pathways involved in mediating the loss of the endothelial barrier (5, 12–16). Thrombin binds to and cleaves protease-activated receptor-1 (PAR-1) in endothelial cells leading to activation of heterotrimeric G-proteins Gαi, Gαi, and G12/13 (17–19). The released α-subunit of G12/13 induces p115RhoGEF activation, a Rho-specific GEF, and thereby activates Rho (20–23). p115RhoGEF contains a Dbh homology (DH) domain as well as pleckstrin homology (PH) domain. The Dbh domain upon binding to Rho induces GDP/GTP exchange, whereas the PH domain acts as a membrane-anchoring domain for RhoGEF. p115RhoGEF structure is complex in that it contains a homologous core regulator of G-protein signaling (RGS) domain at its N-terminal region by which p115RhoGEF interacts with α-subunits of G12/13 and functions as a GTPase-activating protein (GAP) for G12/13. Thus, p115RhoGEF is not only a GAP and G12/13 effector but also a linker protein between G12/13 and the Rho pathway (23–26).

Although G12/13 can directly stimulate the exchange activity of p115RhoGEF on Rho GTPase (20, 23–25, 27), there may be additional regulatory pathways contributing to p115RhoGEF activation. Studies showed that activation of the GTPases, Rac and Cdc42, required the phosphorylation of their respective GEFs, Tiam and Vav (28–30). The Leukemia-associated RhoGEF (LARG), a RhoGEF containing a RGS domain similar to p115RhoGEF, is also activated by phosphorylation (31, 32). We previously showed (14) that PKCa triggered Rho activation and promoted actin cytoskeletal changes in endothelial cells. As it is possible that PKCa may regulate Rho activation by inducing the phosphorylation of p115RhoGEF, the present study was carried out to determine the role of PKCa in signaling p115RhoGEF activation, and in thus triggering Rho-dependent endothelial actin re-arrangement.

EXPERIMENTAL PROCEDURES

Materials—Human α-thrombin was purchased from Enzyme Research Laboratories (South Bend, IN). Human umbilical vein endothelial cells (HUVECs) and endothelial growth medium (EBM-2) were purchased from Clonetics (San Diego, CA). Trypsin was purchased from Life Technologies, Inc. Anti-p115RhoGEF, anti-PKCα, anti-RhoA, normal goat IgG, and protein A/G beads were purchased from Santa Cruz Biotechnology (San Diego, CA). H2PO4 was purchased from ICN Pharmaceuticals (Costa Mesa, CA). Alexa Fluor 568 phalloidin, 4′,6-diamidino-2-phenylindole, dihydrochloride, and Prolong Antifade kit were purchased from Molecular Probes (Eugene, OR). Electrodes for transendothelial electrical resistance measurements were purchased from Applied Biosciences (Troy, NY). G66976 was purchased from Calbiochem (San Diego, CA). Rho activation kit containing GST-rotekin-Rho-binding domain beads was purchased from Cytoskeleton (Denver, CO).

Endothelial Cell Culture—HUVECs were cultured in a T-75 flask coated with 0.1% gelatin in EBM-2 medium supplemented with 10% fetal bovine serum. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO2 and 95% air until they formed a confluent

* This work was supported by National Institutes of Health Grants HLBI T32-HL07239, HL46350, HL46568, and HL71794. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence should be addressed: Dept. of Pharmacology, University of Illinois, College of Medicine, 335 S. Wolcott Ave. (M/C 868), Tel.: 312-355-0236; Fax: 312-996-1225; E-mail: dmehta@uic.edu.

© 2003 by The American Society for Biochemistry and Molecular Biology, Inc.

This paper is available on line at http://www.jbc.org

Vol. 278, No. 31, Issue of August 1, pp. 28793–28798, 2003

Printed in U.S.A.
**PKCa and G_{12}/G_{13} Regulation of 115RhoGEF Activation**

**FIG. 1.** Thrombin induces the phosphorylation of p115RhoGEF in HUVECs. Endothelial cells were incubated for 4 h with 32P-H3PO4 and stimulated with 50 nM thrombin for the indicated time. Cell lysates were immunoprecipitated with p115RhoGEF Ab, electrophoresed, and transferred to nitrocellulose membrane for analysis of phosphorylation and p115RhoGEF content by Western blotting. A, representative autoradiograph and Western blots showing p115RhoGEF phosphorylation after thrombin stimulation of endothelial cells. B, data from multiple experiments are shown as mean ± S.E. (n = 5–7) of % increase in phosphorylation of p115RhoGEF. Increase in phosphorylation was quantified as percent increase over phosphorylation at time 0. *, values different from unstimulated cells (p < 0.05); +, presence; −, absence.

**FIG. 2.** PKCa regulates thrombin-induced p115RhoGEF phosphorylation. 32P-labeled endothelial cells were stimulated with 50 nM thrombin for 1 min. Cell lysates were immunoprecipitated with anti-p115RhoGEF Ab, electrophoresed, and transferred to nitrocellulose membrane for analysis of phosphorylation and p115RhoGEF content by autoradiography and Western blotting. A, representative autoradiograph and Western blots showing p115RhoGEF phosphorylation after thrombin stimulation of cells treated either with or without Go6976 treatment. Increase in phosphorylation was quantified as percent increase over phosphorylation at time 0. B, data from multiple experiments are shown as mean ± S.E. (n = 3) of % increase in phosphorylation of p115RhoGEF in cells either with or without Go6976 treatment. Increase in phosphorylation was quantified as percent increase over phosphorylation at time 0. C, Western blot showing over-expression of PKCa in cells infected with Adv-dnPKCa compared with control cells or cells infected with Adv-LacZ. D, representative autoradiograph and Western blots showing p115RhoGEF phosphorylation in response to thrombin in cells infected with Adv-LacZ or Adv-dnPKCa. Results are representative of at least 2 experiments. *, values different from unstimulated cells (p < 0.05); +, presence; −, absence.

monolayer. Cells from each of the primary flasks were detached with 0.025% Trypsin/EDTA and plated on either 100 mm dishes for phosphorylation analysis, 8-well electrodes for electrical impedance measurements, 6-well plates for serum response element (SRE) luciferase assays, or the cells were grown on coverslips for immunofluorescence microscopy as described below. In all experiments, the monolayer of HUVEC was incubated for 1–2 h in MCDB131 serum-free medium before treatment with inhibitors or agonists. In all experiments, cells were used between passages 4–8. DEAE dextran or Superfect was used for transfection of endothelial cells as specified by the suppliers' protocols.

**Adenoviral Infections**—Adenoviral constructs containing the dominant negative mutant of PKCα (Adv-dnPKCa; K368R) was a generous gift from Dr. Viswanathnan Natarajan (Johns Hopkins University School of Medicine, Baltimore, MD). Cells grown to confluence in 100 mm dishes (phosphorylation and Rho activation) or on 12 mm coverslips (immunohistochemistry) were infected with 1 × 10^10 pfu of Adv-dnPKα for 5 h in serum-containing medium, after which the media was replaced with fresh media. In parallel, monolayers were infected with adenovirus containing the β-galactosidase gene, LacZ (Adv-LacZ), to control for any nonspecific effects of viral infection on endothelial cell signaling. Adv-infected cells were then used to measure p115RhoGEF phosphorylation, Rho activation, and actin stress fiber formation as described below.

**Phosphorylation of p115RhoGEF**—After serum deprivation for 1 h, HUVECs were labeled with 150 μCi/ml 32P for 4 h in phosphate-free minimum Eagle’s medium. Cells were then stimulated with 50 nM α-thrombin for the indicated times, quickly transferred on ice, and washed 2× with ice-cold phosphate-buffered saline containing 500 μM Na3VO4 and lysed using radioimmune precipitation assay buffer (1% Triton-X, 150 mM NaCl, 10 mM Tris, 1 mM EDTA, 2 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride, 0.5% Nonidet P-40, and 2 μg/ml each of pepstatin A, leupeptin, and aprotonin). After centrifugation, the cleared lysate was incubated with either control IgG or anti-gout polyclonal p115RhoGEF Ab for 2 h followed by addition of protein A/G plus agarose beads overnight. The beads were then collected by centrifugation, washed 6× with detergent-free radioimmune precipitation assay buffer (150 mM NaCl, 10 mM Tris, 1 mM EDTA, 0.05% SDS and 2 μg/ml each of pepstatin A, leupeptin, and aprotonin). The above procedures were performed at 4 °C. Protein from each sample was eluted by boiling the beads in SDS sample buffer, electrophoresed on 7.5% SDS-polyacrylamide gels, and transferred to...
nitrocellulose for visualization of p115RhoGEF phosphorylation by autoradiography followed by Western blotting with p115RhoGEF antibody to verify equal protein loading. Specificity of p115RhoGEF antibody was confirmed using normal goat IgG as negative control.

**Immunoprecipitation and Immunoblotting—**Endothelial cells grown to confluence in 100 mm dishes were serum-deprived after which they were stimulated with 50 nM α-thrombin for the indicated times. Cells were then washed 2× with cold phosphate-buffered saline and lysed using radiomimic precipitation assay buffer. Lysates were centrifuged at 14,000 rpm for 10 min at 4 °C, and protein concentration was determined. Cell lysates containing equal amounts of protein (300 μg) were incubated with 3 μg of either control IgG or anti-p115RhoGEF Ab or anti-PKCα Ab. After 2 h, 20 μl of protein A/G beads were added, and the samples were incubated overnight. Each sample was then centrifuged to retrieve the precipitated complex on the beads and washed 4× with wash buffer followed by addition of sample buffer. Proteins from each sample were eluted by boiling the beads, separated by electrophoresis on a 10% polyacrylamide gel, and transferred to nitrocellulose membrane for Western blotting with the antibody of interest.

**Measurement of Rho Activity—**Rho activity was measured using GST-rhotekin-Rho-binding domain (GST-RBD) that specifically pulls down activated Rho (6, 33). Following infection with Adv-constructs as described above, HUVECs were serum-deprived for 1 h. Cells were then stimulated with 50 nM α-thrombin, washed 2× with ice-cold Tris-buffered saline, and lysed in 500 μl of lysis buffer (50 mM Tris pH 7.5, 10 mM MgCl₂, 0.5 M NaCl, 1% Triton X-100, 0.5% Sodium deoxycholate, 0.1% SDS, 500 μg/ml tsoyl arginine methyl ester, 10 μg/ml each of leupeptin and aprotinin). Cell lysates were immediately centrifuged at 8,000 rpm at 4 °C for 5 min and equal volumes of lysates were incubated with 30 μg GST-RBD beads for 1 h at 4 °C. The beads were washed 3× with wash buffer (25 mM Tris pH 7.5, 30 mM MgCl₂, 40 mM NaCl), and bound Rho was eluted by boiling each sample in Laemmli sample buffer. Eluted samples from the beads and total cell lysate were then electrophoresed on 12.5% SDS-polyacrylamide gel electrophoresis gels, transferred to nitrocellulose, blocked with 5% nonfat milk, and analyzed by Western blotting using a polyclonal anti-RhoA antibody.

**Actin Stress Fibers—**Following infection with Adv-constructs or indicated inhibitor, cells were stimulated for 5 min with 50 nM α-thrombin, rinsed quickly with ice cold Hank’s balanced salt solution, and fixed with 4% paraformaldehyde. Cells were permeabilized for 3 min with 0.1% Triton X-100 in Hank’s balanced salt solution followed by incubation for 20 min with bovine serum albumin. Cells were then incubated with Alexa-568 phalloidin to label stress fibers. Following incubation, cells were rinsed 6× with Hank’s balanced salt solution and mounted on slides using Prolong antifade mounting kit. Cells were viewed with a Zeiss LSM-510 confocal microscope using appropriate filters.

**Reporter Gene Constructs, Endothelial Cell Transfection, and Luciferase Assay—**We used SRE reporter gene activity to address the role of p115RhoGEF in inducing Rho activation (28). We used a mutant containing the p115-RGS domain (RGS mutant) to address its role in thrombin-induced Rho activation. Transfections were performed using the DEAE-dextran method as described (14). DNA (2.5 μg of p115-RGS cDNA and 2.5 μg of SRE luciferase cDNA) containing the RGS mutant of p115 and 2.4 μg of SRE luciferase were mixed with 50 μg/ml DEAE-dextran in serum-free MCD131 medium, and the mixture was added onto confluent cells. For determining the role of PKCα-induced p115RhoGEF phosphorylation in signaling Rho activation, the transfected cells were treated with Go6976 for 1 h prior to stimulation. pTKRLUC plasmid (0.1 μg) (Promega Corp., Madison, WI) containing Renilla luciferase gene driven by the constitutively active thymidine kinase promoter was added to normalize the transfection efficiencies. After 1 h, cells were incubated for 4 min with 10% dimethyl sulfoxide (Me2SO) in serum-free medium, washed 2× with EBM-2 containing 10% fetal bovine serum, and grown to confluence. Following 6 h of stimulation with 50 nM α-thrombin, cell extracts were prepared and assayed for luciferase activity using the Dual Luciferase Reporter Assay system (Promega). SRE-luciferase activity was expressed as the ratio of firefly and Renilla luciferase activity.

**Transendothelial Electrical Resistance—**The time course of endothelial cell retraction, a measure of increased endothelial permeability, was assessed according to described procedures (34). Briefly, HUVECs grown to confluence on gelatin-coated small gold electrodes (4.9 × 10⁴ cm²) were serum-deprived for 1 h, after which they were treated with the indicated reagent prior to thrombin stimulation. For studies involving the p115-RGS mutant, endothelial cells grown to 70% confluence were transfected using Superfect transfection reagent following supplier’s protocol. Cells were then stimulated with 50 nM α-thrombin to determine transendothelial electrical resistance across the monolayer. The small electrode and larger counter electrode were connected to a phase-sensitive lock-in amplifier. A constant current of 1 μA was supplied by a 10 V 4 KHz alternating current connected serially to a 1 MΩ resistor between the small electrode and the larger counter electrode. The voltage between the small and large electrode was monitored by a lock-in amplifier, stored, and processed on a PC. Data are presented as percentage change in their resistive (in-phase) portion of impedance normalized to its initial value at time zero.

**RESULTS**

**Thrombin-induced Phosphorylation of p115RhoGEF in Endothelial Cells Is PKCα-dependent—**Cells grown to confluence were labeled with 32P-HPO₄ after which they were stimulated with thrombin, immunoprecipitated with p115RhoGEF Ab, and electrophoresed to determine p115RhoGEF phosphorylation by autoradiography. Thrombin induced the phosphorylation of p115RhoGEF within 1 min, and the response was sustained for 10 min after thrombin challenge (Fig. 1, A and B).

We investigated the possibility that thrombin induces p115RhoGEF phosphorylation in a PKCα-dependent manner. We used Go6976, an inhibitor of PKCα (35), as well as an adenoviral construct containing the kinase-defective mutant of PKCα (Adv-dnPKCα), to address the role of PKCα in thrombin-induced phosphorylation of p115RhoGEF. Fig. 2A shows the autoradiograph of p115RhoGEF phosphorylation in response to

---

**FIG.3.** Thrombin induces the rapid association of PKCα with p115-RhoGEF. HUVECs were stimulated with 50 nM α-thrombin for 1 min, lysed, and cell lysates were immunoprecipitated with either control IgG (A), anti-p115RhoGEF Ab (B), or anti-PKCα Ab (C), and Western blotted with anti-p115RhoGEF, anti-PKCα, or anti-PKCα Abs. +, presence; −, absence. Results are representative of at least 2 experiments.
PKCα and G12/G13 Regulation of 115RhoGEF Activation

**Fig. 4. Thrombin induced Rho activation requires G_{12/13} and PKCα pathway.** A, cells infected with either Adv-LacZ or Adv-dnPKCα were stimulated with thrombin for 1 min after which they were lysed to measure Rho activity. Rho activity is indicated by the amount of RBD-bound Rho (top) normalized to the amount of Rho in whole cell lysate (bottom). Data are representative of 3 independent experiments. B, actin stress fiber formation in cells stimulated with thrombin for 5 min following the indicated treatment. Results are representative of at least 2 experiments. C, SRE production in endothelial cells in response to thrombin stimulation. Cells were co-transfected with SRE-Luc plasmid without or with p115-RGS mutant. Cells were then left untreated or treated with Go6976 for 1 h after which they were stimulated with thrombin for 5 h prior to SRE activity measurement. Data are mean ± S.E. (n = 3 for each condition). SRE-luc activity is expressed as the ratio of firefly and Renilla luciferase activity quantified as fold increase over unstimulated. *, values different from control cells (p < 0.05); +, presence; −, absence; arrows indicate cell over-expressing p115-RGS as represented by GFP transfection marker.

PKCα Phosphorylation of p115RhoGEF Activates Rho—To determine whether the PKCα-induced phosphorylation of p115RhoGEF is capable of signaling Rho activation, we measured Rho activation using the GST-Rho-kinase fusion protein in cells over-expressing Adv-dnPKCα. Following viral infection, cells were stimulated with thrombin, and the lysates from cells infected with Adv-dnPKCα or Adv-LacZ were incubated with Rho-kinase bound to GST beads to determine Rho activation. As shown in Fig. 4A, the inhibition of p115RhoGEF phosphorylation prevented the thrombin-induced Rho activation.

Parallel Regulation of p115RhoGEF-dependent Actin Stress Fiber Formation by PKCα and G_{12/13}—As p115RhoGEF is activated downstream of G_{12/13} (15–18), we determined the contributions of G_{12/13} and PKCα in p115RhoGEF activation and in the mechanism of thrombin-induced Rho activation and actin cytoskeletal re-arrangement. Expression of p115-RGS inhibits endogenous p115RhoGEF function by blocking the interaction of p115RhoGEF with α-subunits of G_{12/13} and by its GAP activity on G_{12/13} (20, 23–26, 36, 37). We used the N-terminal RGS domain of p115RhoGEF (p115-RGS), which inhibits p115RhoGEF function by blocking the interaction of p115-RhoGEF with the α-subunits of G_{12/13} and by its GAP activity on G_{12/13}, to address the role of G_{12/13}-induced Rho activation in endothelial actin cytoskeletal alterations. Thrombin phosphorylation of p115RhoGEF (Fig. 3).

Association of PKCα with p115RhoGEF—To determine whether thrombin-induced regulation of p115RhoGEF by PKCα required their interaction, cell lysates were immunoprecipitated with either control IgG, anti-p115RhoGEF Ab, or anti-PKCα Ab. Immunoprecipitated samples were then separated by SDS-PAGE and Western blotted with anti-p115RhoGEF, anti-PKCα, or PKCα Abs. As shown in Fig. 3, thrombin induced the association of PKCα with p115RhoGEF regardless of whether lysates were immunoprecipitated with anti-PKCα or anti-p115RhoGEF Abs. In contrast under similar conditions, PKCε and control goat IgG showed no interaction with p115RhoGEF (Fig. 3).
induced the formation of actin stress fibers in cells infected with Adv-LacZ (Fig. 4B). However, thrombin failed to induce stress fiber formation in cells when PKC\(\beta\)/H9251 function was inhibited by treatment with Go\(\beta\)6976 or after over-expression of dnPKC\(\beta\)/H9251 (Fig. 4B). Inhibition of p115RhoGEF function by over-expressing the p115-RGS mutant also blocked thrombin-induced stress fiber formation. Thrombin induced a marked increase in SRE generation in control cells indicative of Rho activation, whereas this response was attenuated in the absence of PKC\(\beta\) or G\(\alpha\)12/13 activation (Fig. 4C).

**DISCUSSION**

Rho is activated by the G\(\alpha\)12/13 family of heterotrimeric GTP-binding proteins through the stimulation of GEF activity of p115RhoGEF (23–25). Using the N-terminal RGS domain of p115RhoGEF, which inhibits endogenous p115RhoGEF function by blocking its interaction with the \(\alpha\)-subunits of G\(\alpha\)12/13 and by its GAP activity on G\(\alpha\)12/13, we show that thrombin activates Rho-dependent signaling and the endothelial permeability response via the G\(\alpha\)12/13 pathway. However, our results demonstrate the existence of another equally important parallel pathway activated by PKC\(\beta\) that also regulates p115RhoGEF function. We show that p115RhoGEF is phosphorylated within 1 min of thrombin stimulation. The phosphorylation of p115RhoGEF is mediated by PKC\(\beta\) as is evident by the experiments employing the PKC\(\beta\)-specific inhibitor Go\(\beta\)6976 and expression of the kinase-defective PKC\(\beta\) mutant. PKC\(\beta\) was found to interact with p115RhoGEF after thrombin stimulation, implying that association of the two may be required for p115RhoGEF activation. We also show that PKC\(\beta\)-dependent phosphorylation of p115RhoGEF plays an important functional role in Rho signaling because inhibition of the phosphorylation prevented the Rho activation as well as actin stress fiber formation and endothelial barrier disruption responses. Taken together, these findings demonstrate an important role of PKC\(\beta\)-induced p115RhoGEF phosphorylation in the mechanism of activation of the Rho pathway in endothelial cells and its downstream effects on the actin cytoskeletal and endothelial barrier function.

It is known that the GTP-binding/GTPase cycle activated by p115RhoGEF regulates Rho activation (38). p115RhoGEF is a specific RhoGEF for Rho (20, 24, 37); however, little is known about the regulation of p115RhoGEF beyond that it is controlled by G\(\alpha\)12/13. The activation of p115RhoGEF involves its translocation from the cytosol to membrane as shown in NIH3T3 and COS-7 cells in response to lysophosphatidic acid stimulation. Studies showed that thrombin-induced Rho acti-
p115RhoGEF within 1 min of thrombin stimulation suggests that both components rapidly interact to induce p115RhoGEF phosphorylation.

Because PKCα is a downstream effector of Gαq, our data suggest that the response is the result of Gαq-activated signaling. This result is consistent with the concept of dual regulation of p115RhoGEF that involves both the Gαq-PKCα and the Gα12/13 pathways. Thus, p115RhoGEF is positioned at the confluent point of these pathways because the activation of p115RhoGEF by either PKCα or Gα12/13 is sufficient to signal Rho activation and induce actin stress fiber formation and increase endothelial permeability.

Among several GEFs identified, p115RhoGEF has the capability of not only activating but also inhibiting Rho activation following stimulation of G-protein-coupled receptors. p115RhoGEF contains an RGS domain at its N terminus through which it interacts with Gα12/13 (20, 26), thus providing a direct link between the Gα12/13-coupled receptor stimulation and Rho activity. The RGS sequence inactivates Gα12/13 signaling via its GAP activity (26). In the present study, we addressed the role of the RGS domain in thrombin-induced endothelial barrier dysfunction. We observed that the effects of expressing the RGS sequence of p115RhoGEF on endothelial barrier function were similar to that observed upon inhibition of PKCα and Gα12/13-coupled receptor stimulation—raising the possibility that p115RhoGEF is a downstream effector of Gα12/13 pathways. Thus, p115RhoGEF is positioned at the confluence point of these pathways because the activation of p115RhoGEF by either PKCα or Gα12/13 is sufficient to signal Rho activation and induce actin stress fiber formation and increase endothelial permeability.

Acknowledgment—The Adv-PKCα adenovirus was kindly supplied by Dr. Viswanathan Natarajan (Johns Hopkins University, Baltimore, MD).

REFERENCES

1. Lum, H., and Malik, A. B. (1996) Can. J. Physiol. Pharmacol. 74, 787–800
2. Dudzik, S. M., and Garcia, J. G. (2001) J. Appl. Physiol. 91, 1467–1500
3. Aepfelbacher, M., and Eessler, M. (2001) Cell Microbiol. 3, 649–658
4. van Nieuw Amerongen, G. P., and van Hinsbergh, V. W. (2001) Arterioscler. Thromb. Vasc. Biol. 21, 300–311
5. van Nieuw Amerongen, G. P., van Delft, S., Vermeer, M. A., Collard, J. G., and van Hinsbergh, V. W. (2000) Circ. Res. 87, 335–340
6. Klages, B., Brandt, U., Simon, M. I., Schulte, C., and Offermanns, S. (1999) J. Cell Biol. 144, 745–754
7. Sah, V. P., Seasholtz, T. M., Sagi, S. A., and Brown, J. H. (2000) Annu. Rev. Pharmacol. Theraput. 40, 459–489
8. Wojciech-Stohard, B., Potempa, S., Eichholtz, T., and Ridley, A. J. (2001) J. Cell Sci. 114, 1343–1355
9. Ridley, A. J. (2001) Trends Cell Biol. 11, 471–477
10. Kranebusch, O., Poland, M., van Hoek, F. P., Drecueil, D., Hall, A., and Moolenar, W. H. (1999) Mol. Biol. Cell. 10, 1851–1857
11. Hall, A. (1998) Science 279, 509–514
12. Tiruppathi, C., Lum, H., Andersen, T. T., Fenton, J. W., 2nd, and Malik, A. B. (1999) Am. J. Physiol. 263, L505–601
13. Lum, H., Andersen, T. T., Sifflinger-Birnboim, A., Tiruppathi, C., Goligorsky, M. S., Fenton, J. W., II, and Malik, A. B. (1992) J. Biol. Chem. 267, 22614–22620
14. Mehta, D., Rahman, A., and Malik, A. B. (2001) J. Biol. Chem. 276, 2223–2229
15. Sandoval, R., Malik, A. B., Naqui, T., Mehta, D., and Tiruppathi, C. (2001) J. Biol. Chem. 276, 1239–1247
16. Mehta, D., Tiruppathi, C., Sandoval, R., Minshall, R. D., Holinstat, M., and Malik, A. B. (2002) J. Physiol. 539, 779–789
17. Coughlin, S. R. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 11023–11027
18. Hung, D. T., Wong, Y. H., Yu, T. K., and Coughlin, S. R. (1992) J. Biol. Chem. 267, 20831–20834
19. Barr, A. J., Brass, L. F., and Manning, D. R. (1997) J. Biol. Chem. 272, 2223–2229
20. Hart, M. J., Jiang, X., Konzasa, T., Roscoe, W., Singer, W. D., Gilman, A. G., Sternweis, P. C., and Bollag, G. (1998) Science 280, 2112–2114
21. An, S., Goetzl, E. J., and Lee, H. (1998) J. Cell. Biochem. Suppl. 30–31, 147–157
22. Bhattacharyya, R., and Wedegaertner, P. B. (2000) J. Biol. Chem. 275, 14992–14999
23. Wells, C. D., Liu, M. Y., Jackson, M., Gutowski, S., Sternweis, P. M., Rothstein, J. D., Konzasa, T., and Sternweis, P. C. (2002) J. Biol. Chem. 277, 1114–1118
24. Wells, C., Jiang, X., Gutowski, S., and Sternweis, P. C. (2002) Methods Enzymol. 345, 371–382
25. Wells, C. D., Gutowski, S., Bollag, G., and Sternweis, P. C. (2001) J. Biol. Chem. 276, 28897–28905
26. Konzasa, T., Jiang, X., Hart, M. J., Sternweis, P. M., Singer, W. D., Gilman, A. G., Bollag, G., and Sternweis, P. C. (1998) Science 280, 2109–2111
27. Hart, M. J., Roscoe, W., and Bollag, G. (2000) Methods Enzymol. 325, 61–71
28. Heuner, S. P., Hofmann, T. G., Dienz, O., Droge, W., and Schmitz, M. L. (2000) J. Biol. Chem. 275, 18160–18171
29. Tamas, P., Solti, Z., and Boday, L. (2001) Cell Signal. 13, 475–481
30. Fleming, I. N., Elliott, C. M., Collard, J. G., and Exton, J. H. (1997) J. Biol. Chem. 272, 33105–33110
31. Suzuki, N., Nakamura, S., Mano, H., and Konzasa, T. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 723–728
32. Chikummi, H., Fukuhara, S., and Gutzkin, J. S. (2002) J. Biol. Chem. 277, 12463–12473
33. Rood, J. D., Kisses, W. B., and Schwartz, M. A. (1999) EMBO J. 18, 578–585
34. Tiruppathi, C., Malik, A. B., De Vecchio, P. J., Keese, C. R., and Giaever, I. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7919–7923
35. Martiny-Baron, G., Kazanietz, M. G., Mischak, H., Blumberg, P. M., Koeh, G., Hug, H., Marme, D., and Schachtel, C. (1993) J. Biol. Chem. 268, 9194–9197
36. Chen, Z., Wells, C. D., Sternweis, P. C., and Sprang, S. R. (2001) Nat. Struct. Biol. 8, 805–809
37. Hart, M. J., Sharma, S., el Masry, N., Qiu, R. G., McCabe, P., Polakis, P., and Bollag, G. (1996) J. Biol. Chem. 271, 25452–25458
38. Konzasa, T. (2001) Life Sci. 68, 2309–2317
39. Deleted in proof
Protein Kinase Cα-Induced p115RhoGEF Phosphorylation Signals Endothelial Cytoskeletal Rearrangement

Michael Holinstat, Dolly Mehta, Tohru Kozasa, Richard D. Minshall and Asrar B. Malik

J. Biol. Chem. 2003, 278:28793-28798.
doi: 10.1074/jbc.M303900200 originally published online May 16, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M303900200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 38 references, 24 of which can be accessed free at http://www.jbc.org/content/278/31/28793.full.html#ref-list-1