PKA inhibits RhoA activation: a protection mechanism against endothelial barrier dysfunction

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Qiao, Jing, Fei Huang, and Hazel Lum. PKA inhibits RhoA activation: a protection mechanism against endothelial barrier dysfunction. Am J Physiol Lung Cell Mol Physiol 284: L972–L980, 2003. First published February 14, 2003; 10.1152/ajplung.00429.2002.—Much evidence indicates that cAMP-dependent protein kinase (PKA) prevents increased endothelial permeability induced by inflammatory mediators. We investigated the hypothesis that PKA inhibits Rho GTPases, which are regulator proteins believed to mediate endothelial barrier dysfunction. Stimulation of human microvascular endothelial cells (HMEC) with thrombin (10 nM) increased activated RhoA (RhoA-GTP) within 1 min, which remained elevated approximately fourfold over control for 15 min. The activation was accompanied by RhoA translocation to the cell membrane. However, thrombin did not activate Cdc42 or Rac1 within similar time points, indicating selectivity of activation responses by Rho GTPases. Pretreatment of HMEC with 10 μM forskolin plus 1 μM IBMX (FI) to elevate intracellular cAMP levels inhibited both thrombin-induced RhoA activation and translocation responses. FI additionally inhibited thrombin-mediated dissociation of RhoA from guanine nucleotide dissociation inhibitor (GDI) and enhanced in vivo incorporation of 32P by GDI. HMEC pretreated in parallel with FI showed >50% reduction in time for the thrombin-mediated resistance drop to return to near baseline and inhibition of ~23% of the extent of resistance drop. Infection of HMEC with replication-deficient adenovirus containing the protein kinase A inhibitor gene (PKA inhibitor) blocked both the FI-mediated protective effects on RhoA activation and resistance changes. In conclusion, the results provide evidence that PKA inhibited RhoA activation in endothelial cells, supporting a signaling mechanism of protection against vascular endothelial barrier dysfunction. Rho guanosine 5’-triphosphate; protein kinase A inhibitor; guanine nucleotide dissociation inhibitor; endothelial resistance

INCREASED VASCULAR PERMEABILITY is a hallmark of inflammation that occurs in acute and chronic diseases such as atherosclerosis, acute respiratory distress syndrome, and diabetes. Inflammatory mediators (i.e., thrombin, TNF-α, transforming growth factor-β) and oxidants activate a repertoire of signaling events in endothelium that results in the development of gaps between cells, leading to barrier dysfunction (17, 19, 25, 34, 35, 39). The cAMP-dependent protein kinase (PKA) has significant and profound protective actions against increases in endothelial permeability. We and others (4, 10, 32, 42, 43) have shown that the ubiquitous cellular messenger cAMP prevents increases in endothelial permeability in response to a wide range of inflammatory mediators, including oxidants. Although growing evidence indicates that cAMP activates both PKA-dependent and -independent actions (8, 11, 26), our recent observations show that protective actions of cAMP against barrier dysfunction are likely mediated predominantly through PKA-dependent signaling mechanisms (32, 43).

Despite the abundant reports documenting cAMP/PKA signaling in inhibition of increases in permeability, the specific mechanisms by which this occurs remain unclear and controversial. One proposed hypothesis suggests that PKA regulates the phosphorylation of myosin light chain (MLC). The phosphorylation of MLC in endothelial cells is mediated primarily by MLC kinase (MLCK), and subsequent MLC-mediated contractions provide the critical mechanical tension in promoting intercellular gap formation that leads to increases in endothelial permeability (20, 33). Endothelial-specific MLCK has been shown to contain PKA consensus phosphorylation sites, and cAMP has been reported to inhibit in vitro MLCK activity (18). Yet, increased intracellular cAMP appears unable to prevent thrombin-induced increases in MLC phosphorylation or contraction of endothelial cells (38), suggesting that protective mechanisms of PKA may not be a direct reversal of MLC phosphorylation and, therefore, of contraction.

Another class of critical regulatory proteins in regulation of endothelial barrier function is the family of Rho GTPases (2, 9, 14, 36, 45, 47). The family consists of 20 distinct members [Rho (A, B, C); Rac1, 2, 3; Cdc42; TC10; TCL; Chp (1, 2); RhoG; Rnd (1, 2, 3); RhoBTB (1, 2); RhoD; Rif; and TTF] (15), of which Cdc42, Rac1, and RhoA are the most characterized (6) and are essential in the relay of signals to the actin cytoskeleton in regulation of activities such as cell adhesion, motility, cell cycle progression, and apoptosis. Current findings suggest that RhoA may regulate multiple targets that may be crucial determinants of endothelial barrier function, including adherens junctures.
Materials and Methods

Materials. The following reagents were purchased as follows: DH5α competent cells, MCDB-131 medium, penicillin-streptomycin, l-glutamine, sodium pyruvate, phosphate-buffered saline (PBS), MEM nonessential amino acids, and MEM vitamins from GIBCO-BRL (Gaithersburg, MD); human endothelial phenotypic, taken up acetylated low-density lipoprotein (LDL), forms tubes when grown in Matrigel, and expresses CD31 and factor, takes up acetylated low-density lipoprotein (LDL), exhibits the expected morphological and functional endothelial phenotypes: it expresses and secretes von Willebrand factor, exhibits the expected morphological and functional endothelial phenotypes: it expresses and secretes von Willebrand factor, exhibits the expected morphological and functional endothelial phenotypes: it expresses and secretes von Willebrand factor, exhibits the expected morphological and functional endothelial phenotypes: it expresses and secretes von Willebrand factor, exhibits the expected morphological and 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GTPases and total Rho GTPases. The membrane was incubated with the appropriate secondary HRP-conjugated antibodies for enhanced chemiluminescence detection. The amount of GTP-bound Rho was quantified by scanning densitometry and normalized to total Rho (GTP-bound + GDP-bound forms) in cell lysates.

***Immunofluorescent confocal microscopy.*** Endothelial cells were plated on glass chamber slides coated with 1 μg/ml of fibronectin and grown overnight. The cells were washed with PBS and fixed with 4% paraformaldehyde in PBS for 10 min at room temperature. The fixed cells were permeabilized with 0.2% Triton X-100 in PBS for 10 min at room temperature, incubated with the appropriate primary antibodies directed against RhoA, Cdc42, or Rac1 for 1 h at room temperature followed by an additional 1-h incubation with biotinylated secondary antibodies, and detected with streptavidin conjugated with Cy2 (Jackson ImmunoResearch Laboratory, West Grove, PA). Image analysis was performed using an Olympus Confocal Fluoview microscope equipped with argon laser (Olympus America, Melville, NY).

***Immunoprecipitation.*** Confluent HMEC grown in 100-mm dishes were serum starved overnight and treated according to experimental protocol. The cells were then quickly washed with ice-cold PBS and lysed in radioimmune precipitation buffer [50 mM Tris (pH 8), 150 mM NaCl, 1% Nonidet P-40, 1 mM EGTA, 1 mM EDTA, 1 mM orthovanadate, 50 mM NaF] plus protease inhibitor cocktail (10 μg/ml of pepstatin A, 10 μg/ml each of aprotonin and leupeptin, and 1 mM PMSP). The cell lysate was passed through a 21-gauge needle eight times, centrifuged at 4°C at 10,000 g for 10 min, and precracked by incubation with 1 μg of normal rabbit IgG with 10 μl of protein A/G plus Agarose. The supernatant was transferred to fresh tubes, incubated with 1.6 μg of rabbit anti-GDI antibody for 1 h at 4°C, then 20 μl of protein A/G plus Agarose was added, and it incubated overnight at 4°C on a rocker platform. The immunoprecipitated protein complex was collected by centrifugation at 2,500 rpm at 4°C for 5 min, washed four times with PBS, boiled in 1× electrophoresis sample buffer, and separated by SDS-PAGE. Western blot analysis was made using anti-RhoA or anti-GDI antibodies to determine coimmunoprecipitation of RhoA with GDI. As negative control, a separate group of cells was used for immunoprecipitation without the precipitating antibody.

***Phosphorylation of Rho-GDI.*** Serum-starved confluent monolayers of HMEC grown in 60-mm dishes were preloaded with 200 μCi/ml [32P]orthophosphate overnight in phosphate-free medium, after which the HMEC were treated according to experimental protocol. The cells were quickly rinsed twice with ice-cold PBS and lysed for 20 min on ice with 300 μl of radioimmune precipitation buffer plus protease inhibitor cocktail and then immunoprecipitated with anti-GDI antibody (as described in ***Immunoprecipitation***). The protein complexes were transferred to nitrocellulose membrane, and autoradiogram was made by exposure to Kodak X-OMAT X-ray film at −80°C. Western blot was made with anti-GDI antibody to determine equal protein loading. As negative control, a separate group of cells was without the precipitating antibody.

***Transendothelial electrical resistance.*** The transendothelial electrical resistance, which provides an index of endothelial barrier function, was measured in real time using the electric cell-substrate impedance sensor (ECIS) system, which detects cell impedance changes in a highly sensitive manner (Applied BioPhysics, Troy, NY) (21, 31, 39). The system consists of a large gold-plated electrode (0.15 cm²) and smaller gold-plated electrodes (5 × 10⁻⁴ cm²) with a 500-μl well fitted above each small electrode. The smaller electrode allows the impedance of the electrode-electrolyte interface at 4,000 Hz to predominate over the solution resistance. The small and large counter electrodes are connected to a phase-sensitive lock-in amplifier, and an alternating current is supplied through the 1-MΩ resistor. The measured electrical impedance indicates the restriction of current flow through the cell monolayer between the electrodes and thus provides an index of the endothelial barrier function.

For resistance measurement, HMEC (2.5 × 10⁶ cells/cm²) were plated onto a sterile, fibronectin-coated, eight-well, gold-plated electrode array and grown to confluence. After being changed to fresh medium, the electrode array was mounted onto holders of the ECIS system housed within an incubator (maintained at 37°C, 5% CO₂, and 100% humidity) and connected to the lock-in amplifier. Voltage and phase data were stored and processed in a computer, which also controlled the output of the amplifier and relay switches to different electrodes. After equilibration for 15–30 min within the incubator, baseline resistance was recorded for another 15–30 min. HMEC monolayers typically show baseline resistance >7,000 Ω (31); therefore, monolayers with lower resistance were rejected from study. We used the minimum baseline resistance recorded for at least 1 h as a reliable control value and normalized all data to the baseline resistance from each experiment.

***Infection with adenovirus containing protein kinase A inhibitor.*** An E1−, E3− replication-deficient adenovirus containing full-length protein kinase A inhibitor (PKI) cDNA (AdPKI) was constructed and characterized as described previously (32). Confluent monolayers of HMEC were infected with AdPKI at 100 multiplicities of infection (MOI) (MOI = plaque-forming units/target cell) for 2 days, and the cells were treated according to experimental protocol. The optimal infection protocol for HMEC has been determined in previous studies (31, 32). Heat-inactivated AdPKI served as control virus as described (32).

***Statistics.*** Single sample data were analyzed by the two-tailed t-test. A multiple range test (Scheffe’s test) was used for comparison of experimental groups with a single control group (44).

**RESULTS**

***Regulation of transendothelial resistance by PKA.*** Transendothelial electrical resistance studies were made to evaluate the regulation of the HMEC barrier function by PKA. Results indicated that thrombin (10 nM) stimulation of HMEC induced rapid decreases in resistance, which reversed to near baseline levels with time (41.9 ± 2.8 min; Fig. 1). Forskolin and IBMX (FI) pretreatment for 15 min resulted in an initial baseline resistance increase and prevented ~23% of the thrombin-induced decreased resistance (Fig. 1B). FI pretreatment also decreased time required for the resistance drop to recover to near baseline (16.2 ± 1.0 min; Fig. 1C). Infection with recombinant AdPKI to overexpress PKI in HMEC prevented the FI-mediated protective effects on the thrombin-induced resistance changes (Fig. 1). The results confirm our previous studies that the protective actions of cAMP against endothelial barrier dysfunction by PKA are dependent on PKA signaling. For references, please see Coronsky et al. (32).
Forskolin (10 μM) and IBMX (1 μM) (FI; arrow) for 15 min to increase intracellular cAMP levels and were then stimulated by thrombin (Thr; 10 nM for 15 min; arrow). Control (C) received buffer challenge.

Results from the studies indicated that PKI overexpression prevented the inhibitory effects of FI on thrombin-induced RhoA activation, restoring ~75% of the thrombin-activated RhoA response (Fig. 4). Infection of HMEC with control heat-inactivated AdPKI was ineffective in blocking the FI inhibitory effect on RhoA activation (data not shown).

The RhoA activation response was additionally evaluated by RhoA translocation to the membrane. The membrane translocation was quantified from randomly selected 20–25 cells/group. The cell membrane area was selected on the basis of predetermined x and y coordinates on the microscope stage at a constant magnification and fluorescent intensity. With the use of confocal system software, the membrane area was outlined and fluorescent intensity quantified. Results were presented as means ± SD from three separate studies. Stimulation of HMEC with thrombin (10 nM for 15 min) increased RhoA fluorescence at the cell membrane 65% over control, indicating RhoA translo-
cation to membrane. In HMEC pretreated with FI, the thrombin-induced increased translocation was reduced by >50% (Fig. 7). These results are consistent with the affinity-binding assay studies showing that the thrombin-induced activation of RhoA was inhibited by cAMP.

We investigated possible mechanisms by which PKA may signal the inhibition of RhoA activation. One possible mechanism is through modulation of GDI, an important regulator of GTP/GDP cycling. GDI inhibits Rho GTPases by binding to the GDP-bound form of Rho to maintain it in a cytoplasmic inactive state. We determined the effects of FI pretreatment of HMEC on GDI binding with RhoA by coprecipitation studies. Results indicated that HMEC stimulated by thrombin (10 nM for 15 min) showed decreased coprecipitation of RhoA with GDI (Fig. 8). Pretreatment with FI prevented this decrease of the coprecipitated complex (Fig. 8), suggesting inhibition of the thrombin-induced dissociation of RhoA from GDI. FI treatment alone showed no change of RhoA coprecipitation with GDI relative to control, whereas the negative control

![Fig. 2. Subcellular localization of RhoA, Cdc42, and Rac1 in endothelial cells. The intracellular distribution of Rho GTPases in HMEC and human pulmonary artery endothelial cells (HPAEC) was evaluated by immunofluorescent laser confocal microscopy. Both endothelial cell types (HMEC: top; HPAEC: bottom) express RhoA, Cdc42, and Rac1. RhoA was found predominantly in the cytosol with some localization in the nucleolus. Cdc42 was distributed in the cytosol and at cell periphery. Rac1 was distributed diffusely in the cytosol and in the nucleus. Original magnification, ×40. Scale bar = 25 μm; n = 3.](image)

![Fig. 3. Effects of thrombin on activation of Rho GTPases. Rho GTPase activation was determined by affinity binding of Rho target fusion proteins (see MATERIALS AND METHODS). HMEC were serum starved and treated with thrombin (10 nM) at 0, 1, or 5 min. The activated (GTP-bound form) and total Rho (Rho-GTP + Rho-GDP) are shown for RhoA (top), Rac1 (middle), and Cdc42 (bottom). Three representative determinations are shown.](image)

![Fig. 4. Effects of PKI overexpression on RhoA activation. HMEC were pretreated with forskolin (10 μM) and IBMX (1 μM) for 15 min to increase intracellular cAMP levels and were then stimulated by thrombin (10 nM for 15 min). Control received buffer challenge. In a separate group, HMEC were infected with 100 MOI AdPKI or control heat-inactivated AdPKI for 2 days before FI and thrombin treatment. RhoA activation was determined by affinity-binding assay as described in MATERIALS AND METHODS. Bar graph summarizes results from 4–6 separate determinations. The Western blot of the affinity binding shows a representative result. RhoA-GTP, activated RhoA; RhoA Total, RhoA-GTP + RhoA-GDP. *P < 0.05 and **P < 0.01 vs. control group.](image)
showed absence of bands. The subsequent stripping of the membrane and detection with anti-GDI antibody indicated equal loading of proteins among groups. We also determined whether the FI-mediated inhibition of RhoA activation was associated with changes in the phosphorylation of GDI. The autoradiographic determination of in vivo incorporation of $^{32}$P by GDI in HMEC indicated that thrombin stimulation alone (10 nM, 15 min) phosphorylated a band at $\sim$30 kDa, corresponding to the molecular weight of GDI (Fig. 9). FI pretreatment enhanced this thrombin-induced phosphorylation of GDI (Fig. 9). The negative control showed absence of phosphorylated bands.

**DISCUSSION**

The critical findings from this study show that pretreatment of HMEC with cAMP-elevating agents 1) inhibited thrombin-induced RhoA activation and translocation to membrane; 2) prevented the thrombin-induced dissociation of RhoA from GDI; and 3) facilitated recovery of the thrombin-mediated resistance decrease and partially inhibited the extent of resistance drop. Infection of HMEC with the recombinant AdPKI blocked the cAMP-mediated protective effects on RhoA activation and resistance changes. The results provide evidence that PKA inhibited RhoA activation in endothelial cells and support a signaling mechanism of protection against vascular endothelial barrier dysfunction.

Our results indicated that thrombin stimulation of human microvascular endothelial cells increased the GTP-bound form of RhoA and RhoA translocation to...
cytes by direct phosphorylation of the COOH terminus served to inhibit RhoA activation in human lympho-
sulting in inhibition of chemoattractant-induced RhoA intracellular cAMP inhibited GTP/GDP exchange, re-
elevation of the responses are translocated to the membrane that is dependent on a posttranslational isoprenylation step (1, 37). This finding provides further support for previous studies in which human umbilical vein endothelial cells (HUVEC) were stimulated by thrombin, resulting in a rapid (<1 min) increase in RhoA activation (36, 45). Several pieces of evidence now implicate RhoA in the regulation of mediator-induced increases in endothelial permeability (9, 14, 36, 40, 45, 47).

Our identification of RhoA as a target for negative regulation by PKA provides a potentially critical mechanism by which the PKA signaling pathway is known to be protective against vascular endothelial barrier dysfunction in response to a wide range of inflammatory mediators. The inhibition of RhoA by the cAMP/PKA pathway has been observed in nonendothelial cells. In a mouse lymphoid cell line, the elevation of intracellular cAMP inhibited GTP/GDP exchange, resulting in inhibition of chemotractant-induced RhoA activation and cell adhesion (28). PKA was also observed to inhibit RhoA activation in human lymphocytes by direct phosphorylation of the COOH terminus at Ser^388, allowing the GTP-bound form of RhoA to be complexed with GDI, thereby preventing translocation to membrane (27). This phosphorylation did not alter the ability of RhoA to bind GTP, nor did it modify its intrinsic GTPase activity. These authors hypothesized that the PKA-mediated phosphorylation of RhoA supports an alternative pathway to terminate RhoA-GTP signaling independent of GTP/GDP cycling (27). However, in endothelial cells, despite the finding that elevation of cAMP inhibited lipopolysaccharide-induced MLC phosphorylation, RhoA was not phosphorylated (14). However, RhoA activity was not measured in this report and, therefore, it is not known whether cAMP inhibited activation of RhoA under these experimental conditions (14). Nonetheless, these observations suggest that PKA signaling may involve both direct and indirect mechanisms of regulation of Rho GTPases. The mechanisms by which inhibition of RhoA activation prevent endothelial barrier dysfunction may be through regulation of several targets critical for the regulation of endothelial barrier function. For example, the expression of dominant negative RhoA in endothelial cells was reported to prevent mediator-induced disassembly of both adherens and tight junctions (47). In one study, inhibition of RhoA resulted in inhibition of increased permeability and phosphorylation of the tight junction protein occludin (24). These studies suggest that junctional proteins may be direct targets of regulation by Rho GTPases. Furthermore, several pieces of evidence document RhoA in the inhibition of myosin-associated protein phosphatases, leading to increased MLC phosphorylation and increased permeability (5, 13, 41). However, Moy and coworkers (38) observed that increased intracellular cAMP was unable to prevent thrombin-induced increases in MLC phosphorylation or contraction of endothelial cells, suggesting that the protective effects of the cAMP/PKA pathway may not be through regulation of MLC phosphorylation. Rho GTPases have also been implicated in the activation of protein kinase C (PKC) (23), an enzyme known to signal increases in endothelial permeability (17, 34, 35).

Because the turning on and off of Rho GTPases are tightly regulated by accessory proteins GDI, GEF, and GAP, we investigated whether PKA-mediated inhibition of RhoA activation involved regulation of one of these proteins, GDI. Our results showed that cAMP-elevating agents prevented the thrombin-induced dissociation of RhoA from GDI, suggesting enhanced stabilization of the Rho-GDI protein complex. GDI is an ubiquitously expressed protein that has been shown to form a complex with different Rho family members, such as RhoA, Rad, Rac2, or CDC42Hs, to maintain them in the inactive state in the cytoplasm (22). Our immunofluorescent observation that cAMP prevented the thrombin-induced RhoA translocation to the membrane is consistent with this thesis. This finding suggests that the RhoA-GDI complex was important in maintaining RhoA in an inactive state in the endothelial cell cytoplasm.

We found that thrombin induced phosphorylation of GDI in endothelial cells. This finding supports the observation made by Mehta and coworkers (36), who observed that thrombin phosphorylated GDI in

![Fig. 8. Coprecipitation of guanine nucleotide dissociation inhibitor (GDI) with RhoA. HMEC were serum starved, stimulated with thrombin alone (10 nM) for 15 min, or pretreated with forskolin (10 μM) and IBMX (1 μM) for 15 min and then stimulated with thrombin. Affinity-purified anti-GDI antibody was used for immunoprecipitation, and separated proteins were detected by Western blot analysis using anti-GDI or anti-RhoA antibodies. As negative control (Neg. C), a separate group of cells was without the precipitating antibody; n = 3.](image)

![Fig. 9. Detection of in vivo GDI phosphorylation. HMEC were pre-loaded with [32P]orthophosphate and treated with forskolin (10 μM) and IBMX (1 μM) for 15 min, followed by thrombin stimulation (10 nM) for 15 min. Cell lysates were prepared for immunoprecipitation with anti-GDI antibody, the immunocomplex was separated by SDS-PAGE, and autoradiograms were prepared (top). Western blot was made to detect for GDI to check for equal loading of samples (bottom). As negative control, a separate group of cells was without the precipitating antibody; n = 3.](image)
HUVEC through activation of PKCα, and proposes that the phosphorylation inhibited GDI function, leading to activation of RhoA. Interestingly, in our studies, cAMP did not inhibit the thrombin-mediated phosphorylation of GDI but enhanced the thrombin-mediated phosphorylation, suggesting that PKA-mediated inhibition of RhoA activation (and inhibition of RhoA dissociation from GDI) was not through inhibition of GDI phosphorylation. However, the GDI amino acid sequence contains both PKA and PKC consensus phosphorylation sites, and it is possible that cAMP may inhibit RhoA activation by mediating phosphorylation of GDI at distinct residues responsible for stabilizing GDI association with RhoA. At present, the functional significance of the several phosphorylation sites of GDI remains to be determined.

We found that the protective action of PKA against endothelial barrier dysfunction consisted of reducing >50% of time required for the thrombin-induced resistance decrease to return to normal baseline levels. Furthermore, PKA inhibited ~23% of the thrombin-induced resistance decrease. In an earlier report, Moy and coworkers (38) also reported that cAMP facilitated restoration of the thrombin-induced resistance decrease to baseline in HUVEC; however, cAMP did not inhibit the initial resistance decrease in thrombin-stimulated cells. Overall, these findings suggest that PKA-mediated protection may be primarily through regulation of the recovery phase of the endothelial barrier dysfunction, and these findings provide further support of our earlier observation that PKA signals the prevention of thrombin-induced increases in transendothelial transport of tracer albumin (32).

We also found that overexpression of PKI did not completely prevent the cAMP-mediated facilitation of recovery, implicating involvement of PKA-independent mechanisms in regulation of this phase of barrier dysfunction. Although the cellular functions of cAMP are presumed to occur predominantly through activation of PKA, it has become increasingly apparent that cAMP has PKA-independent actions as well. For example, cAMP can directly activate a family of guanine nucleotide exchange factors, which in turn activates Ras (8) and Rap-1 (11, 26). Ras and Rap-1 are important in mediating cellular processes such as proliferation, differentiation, and gene expression. The precise role of these potential direct targets of cAMP in regulation of endothelial barrier function remains to be determined.

We observe that thrombin activated RhoA, but not Rac1 or Cdc42, in HMEC. Similarly, van Nieuw Amerongen and coworkers (45) reported that thrombin activated RhoA, but not Rac, in HUVEC. These findings suggest that thrombin shows selectivity in the activation of Rho GTPases. It is evident that endothelial cells in general likely express the three primary Rho GTPases, as we found that the subcellular distributions of RhoA, Rac1, and Cdc42 occurred in both HMEC and HPAEC. At present, it is not known whether other inflammatory mediators show similar selectivity in the activation of Rho GTPases. To date, there have been few studies identifying which inflammatory mediators activate Rho GTPases. Studies have mostly investigated the functional effects of inhibition of Rho GTPases by use of bacterial toxins or transgene expression of mutant forms of RhoA, Rac, or Cdc42. Nonetheless, the results from such studies provide some indirect support that other inflammatory mediators, such as TNF-α (46), lipopolysaccharide (14), bradykinin (2), and platelet-activating factor (2), activate Rho GTPases of the vascular endothelium.

In summary, we report that treatment of endothelial cells with cAMP-elevating agents 1) inhibited thrombin-induced RhoA activation and translocation to membrane; 2) prevented the thrombin-induced dissociation of RhoA from GDI; and 3) facilitated recovery of the thrombin-mediated resistance decrease and partially inhibited the extent of resistance drop. Infection of endothelial cells with the recombinant AdPKI to overexpress PKI blocked the cAMP-mediated protective effects on both RhoA activation and resistance changes. The results provide evidence that PKA inhibited RhoA activation in endothelial cells and support a potentially critical signaling mechanism of protection against vascular endothelial barrier dysfunction.

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