Negative Regulation of Rho Signaling by Insulin and Its Impact on Actin Cytoskeleton Organization in Vascular Smooth Muscle Cells

Role of Nitric Oxide and Cyclic Guanosine Monophosphate Signaling Pathways

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Recent studies from our laboratory have shown that insulin induces relaxation of vascular smooth muscle cells (VSMCs) via stimulation of myosin phosphatase and inhibition of Rho kinase activity. In this study, we examined the mechanism whereby insulin inhibits Rho signaling and its impact on actin cytoskeleton organization. Incubation of confluent serum-starved VSMCs with thrombin or phenylephrine (PE) caused a rapid increase in glutathione S-transferase-Rho-kinase-Rho binding domain–associated RhoA, Rho kinase activation, and actin cytoskeleton organization, which was blocked by preincubation with insulin. Preexposure to N4-monomethyl-L-arginine acetate (L-NMMA), a nitric oxide synthase inhibitor, and Rp-8 CPT-cyclic guanosine monophosphate (RpGMP), a cyclic guanosine monophosphate (cGMP) antagonist, attenuated the inhibitory effect of insulin on RhoA activation and restored thrombin-induced Rho kinase activation, and site-specific phosphorylation of the myosin-bound regulatory subunit (MBSThr695)of myosin-bound phosphatase (MBP), and caused actin fiber reorganization. In contrast, 8-bromo-cGMP, a cGMP agonist, mimicked the inhibitory effects of insulin and abolished thrombin-mediated Rho activation. Insulin inactivation of RhoA was accompanied by inhibition of isoprenylation via reductions in geranylgeranyl transferase-1 activity as well as increased inhibition of isoprenylation via reductions in geranylgeranylation and phosphorylation of RhoA via nitric oxide/cGMP signaling pathway to cause MBP activation, actin cytoskeletal disorganization, and vasodilation. Diabetes 51:2256–2263, 2002

The Rho family of small GTPases are well-recognized intracellular signaling proteins that act as molecular switches to control actin cytoskeleton organization in many cell types, including smooth muscle (1–4). In addition, RhoA-dependent signaling pathway controls important vascular smooth muscle cell functions such as contraction, migration, and proliferation (5,6). In vascular smooth muscle cells (VSMCs), the contracting effect of RhoA results from the activation of Rho-dependent kinase (ROK-α), which phosphorylates the regulatory subunit of myosin light chain (MLC) phosphatase (myosin-bound regulatory subunit [MBS]) leading to the inhibition of its function by reductions in the phosphatase activity (7,8), thus allowing an increase in the level of phosphorylated MLC and contraction at a constant intracellular calcium level (7–10).

Activation of Rho GTPases can be regulated by several mechanisms, including the activation of heterotrimeric G-protein–coupled receptors (11). On agonist stimulation, Rho is converted from the inactive guanosine diphosphate–bound form to the active GTP-bound form. Activation of RhoA by the agonists requires translocation of inactive cytosolic RhoA to the membrane fractions. Thus, appearance of RhoA in the membrane fraction is indicative of Rho activation (12). Rho activation and/or membrane localization is regulated by posttranslational modification by geranylgeranylation and phosphorylation of Rho (13–15). Phosphorylation by cAMP-dependent protein kinase (protein kinase A) as well as by cyclic guanosine monophosphate (cGMP)-dependent protein kinase inhibits Rho signaling by interfering with the membrane anchoring of Rho (14–16). Rho signaling pathway has been implicated in the pathogenesis of hypertension as well as diabetes (17–20). However, the agonists and the mechanism that counterregulate Rho signaling in vivo have not been clearly defined.

Recent studies from this laboratory have shown that insulin rapidly stimulates myosin-bound phosphatase (MBP) activity to cause MLC dephosphorylation and relaxation of VSMCs (20,21). MBP activation by insulin was accompanied by a decrease in Rho kinase activity. These
The effects of insulin were severely impaired in VSMCs isolated from diabetic Goto-Kakizaki rats.

Because Rho kinase is one of the downstream targets of RhoA and activation of Rho kinase is dependent on RhoA activation and translocation to membrane fraction, in this study, we have examined the molecular mechanism by which insulin may inhibit Rho signaling and its impact on actin stress fiber organization.

**RESEARCH DESIGN AND METHODS**

**Culture of VSMCs and treatment.** VSMCs were isolated from rat thoracic aortas and maintained in culture as described in our recent publications (20–24). All experiments were performed on highly confluent cultures (7–9 days in culture) at identical passages. Before each experiment, cells were serum starved for 24 h in serum-free α-MEM containing 5.5 mmol/l glucose. The next day, cells were exposed to insulin (0–100 nmol/l) for 0–30 min. In some experiments, VSMCs were pretreated with various inhibitors for 30 min, followed by exposure to insulin as detailed in the figure legends.

**Metabolic labeling of VSMCs and measurement of RhoA phosphorylation by immunoprecipitation and Western blot analyses.** Serum-starved VSMCs labeled with [32P]orthophosphoric acid (0.3 mCi/ml × 4 h) were exposed to various agonists as detailed in the figure legends. The cells were lysed in a buffer containing 50 mmol/l HEPESS, 2 mmol/l EDTA, 1% Triton X-100, and a cocktail of phosphatase and protease inhibitors, pH 7.5 (20–24). Precleared lysates with equal amounts of proteins (200 µg) were immunoprecipitated with anti-RhoA antibody (10 µg) prebound to anti-mouse IgG agarose (100 µl) for 3 h at 4°C followed by separation of the immunoprecipitates by SDS/PAGE and autoradiography. The level of RhoA phosphorylation was measured by densitometric scanning of the autoradiograms. To overcome variations in proteins due to immunoprecipitation, membranes were probed with anti-RhoA antibody followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies and detection by enhanced chemiluminescence (ECL). The extent of RhoA phosphorylation was quantitated by dividing the intensity of radioactive signal with the protein signal.

**Immunoprecipitation and in vitro assay of Rho kinase activity in the immune-complexes.** Rho kinase was immunoprecipitated using anti-Rho-α antibody as detailed in our recent publications (20,21). Rho kinase activity in the immunoprecipitates was assayed using the recombinant MBP as a substrate (20).

**Analyses of agonist-induced Rho translocation to the membrane fraction.** Cytosolic and membrane fractions were prepared by differential centrifugation according to previously published protocols (20,21). Equal amounts of membrane fractions were subjected to SDS/PAGE, transferred to polyvinylidene fluoride membrane, and probed with mouse anti-RhoA antibody followed by incubation with HRP-labeled secondary antibody and subsequent detection with ECL.

**Analysis of RhoA activation by glutathione S-transferase-Rhotekin-Rho binding domain pull down assay.** [32P]-labeled VSMCs were exposed to various agonists as detailed in Fig. 1. Cells were solubilized and equal amounts of precipitated lysates (250 µg) were incubated with 25 µg (40 µl) of the Rhotekin-Rho binding domain (RBD)-agarose slurry (UBI, Lake Placid, NY) and incubated for 1 h at 4°C with shaking. The agarose beads were washed with wash buffer according to the manufacturer’s protocol and resuspended in 20 µl Lammli sample buffer followed by SDS/PAGE and Western blot analysis with RhoA antibody.

**Immunofluorescence studies on actin cytoskeleton organization.** Serum-starved VSMCs were stimulated with thrombin (1 unit/ml) for 30 min or treated first with insulin (100 nmol/l) for 10 min followed by thrombin. In the inhibitor studies, cells were treated with the inhibitors for 60 min before exposure to insulin and thrombin, as detailed in the figure legend. At the end of the incubation, cells were rinsed with PBS, fixed for 10 min with 3.7% formaldehyde in PBS, permeabilized in 0.1% Triton X-100 in PBS for 5 min, and thrice rinsed with PBS. Cells were double stained with DQ-conjugated Texas Red conjugate and fluorescein isothiocyanate (FITC)-conjugated smooth muscle α-actin antibody to colocalize monomeric globular globular (G-actin) and polymerized filamentous actin (F-actin), respectively (25). The chamber slides were examined with an inverted fluorescence microscope (Eclipse TE-300; Nikon). Images were collected with a Sony 3CCD DXC9000 video camera at 100× magnification and digitally imported into Adobe Photoshop 5.0 and analyzed. FITC-actin and Texas Red-DNase I fluorescence were collected. The time of measurements and image capturing and the image intensity gain at both wavelengths were optimally adjusted and kept constant between each treatment. The ratio of fluorescence of FITC-actin and Texas Red-DNase I (F-actin to–G-actin ratio) used to

**RESULTS**

**Insulin inhibits thrombin/phenylephrine-mediated RhoA translocation.** Inhibitors of nitric oxide synthase (NOS) and cGMP pathway abolish insulin-mediated RhoA inactivation. As seen in Fig. 1, a considerable amount of RhoA was present in the membrane fraction under basal conditions. Thrombin (1 unit/ml) or phenylephrine (PE)
(data not shown) treatment for 5 min caused a threefold increase in membrane-associated RhoA content. Pretreatment with 100 nmol/l insulin for 10 min prevented thrombin-mediated increase in membrane RhoA content. Insulin by itself caused a 40% decrease in membrane-associated RhoA when compared with basal RhoA levels. The agonist-mediated increase in membrane-associated RhoA was accompanied by a concomitant decrease in cytosolic RhoA (data not shown). However, this observation was not always consistent, probably because a large fraction of the total Rho protein still remained cytosolic, as previously reported (29).

To evaluate the role of nitric oxide (NO)/cGMP pathway in insulin inhibition of Rho translocation, VSMCs were pretreated with 1 mmol/l N^G-monomethyl l-arginine acetate (l-NMMA) (a NOS inhibitor) and 100 nmol/l Rp-8 CPT-cyclic guanosine monophosphate (Rp-cGMP) (a cGMP antagonist) for 30 min followed by incubation with 100 nmol/l insulin for 10 min and then exposed to thrombin (1 unit/ml) for 5 min. VSMCs were examined for translocation of RhoA and Rho kinase activity in the anti-ROK-α immunoprecipitates. Both l-NMMA and Rp-cGMP prevented the inhibitory effect of insulin on thrombin-induced RhoA translocation (Fig. 1A, lanes 5 and 6 versus lane 4 and Fig. 1B). l-NMMA and Rp-cGMP alone did not significantly alter RhoA content in the membrane fraction (data not shown). Furthermore, exposure of VSMCs to 8-bromo-cGMP, a cGMP agonist, abolished thrombin-mediated RhoA translocation and decreased membrane-associated RhoA content below basal values (Fig. 1A, lane 7 versus lane 3 and Fig. 1B), as seen with insulin treatment (Fig. 1A, lane 4). The concentrations of inhibitors used were determined in preliminary experiments. l-NMMA at 1 mmol/l and Rp-CPT-cGMP at 100 μmol/l were most effective in inhibiting the effect of insulin on Rho, Rho kinase, and MBP. Glut-1 and the α1 subunit of Na^+/K^+ ATPase, used as an internal control for membrane proteins, revealed equal staining in all lanes (data not shown).

**NO and cGMP inhibitors prevent insulin-mediated inactivation of Rho kinase activity.** As detailed in our recent publications (20,21), insulin treatment for 10 min caused a 35% decrease in basal Rho kinase activity (Fig. 2). Thrombin increased Rho kinase activity by 40% over the basal values, an effect that was prevented by preincubation with insulin. Pretreatment with l-NMMA and Rp-cGMP before insulin abolished insulin’s inhibitory effect and restored thrombin’s effect on Rho kinase activity (Fig. 2). These inhibitors alone had very little effect on basal Rho kinase activity. Furthermore, treatment with 8-bromo-cGMP decreased thrombin-stimulated Rho kinase activity to levels below basal values (Fig. 2).

**NO/cGMP pathway mediates insulin-induced decrease in MBSThr695 phosphorylation.** Recent studies have shown that Rho kinase phosphorylates MBS on the specific sites that influence MBP enzymatic activity (28,30). Therefore, we examined the effect of insulin on MBSThr695 phosphorylation. MBS is phosphorylated to a considerable extent at Thr695 in the basal state. Exposure to insulin for 10 min decreased basal MBSThr695 phosphorylation (Fig. 3A, lane 2 and Fig. 3B) and completely abolished the thrombin-mediated increase in MBSThr695 phosphorylation (Fig. 3A, lane 4 versus lane 3 and Fig. 3B). Pretreatment with l-NMMA and Rp-cGMP prevented the insulin-mediated decrease in MBSThr695 phosphorylation and restored the effect of thrombin on MBSThr695 phosphorylation (Fig. 3A, lanes 5 and 6 versus lane 4 and Fig. 3B). In contrast, pretreatment with 8-bromo-cGMP, a cyclic GMP agonist, prevented thrombin-mediated MBSThr695 phosphorylation in a manner comparable to insulin (Fig. 3A, lane 7 and Fig. 3B). These inhibitors alone had very little effect on basal MBSThr695 phosphorylation (Fig. 3A, lanes 8 and 9 and Fig. 3B). The alterations in MBSThr695 phosphorylation were accompanied by reciprocal changes in MBP activation. Thus, the insulin-mediated reduction in MBSThr695 phosphorylation was accompanied by a 65% increase in MBP activity over the basal values. Moreover, insulin prevented the inhibitory effects of thrombin on MBP activation (Fig. 3C). Both l-NMMA and Rp-cGMP, which increased MBSThr695 phosphorylation, prevented the stimulatory effect of insulin on MBP activation (Fig. 3C).

**Insulin inhibits Rho signaling by altering posttranslational modification of RhoA via the NO/cGMP signaling pathway.** Recent studies indicate that phosphorylation of RhoA by cAMP-dependent protein kinase A as well as by cGMP-dependent protein kinases impairs its biological activity (13,14), whereas geranylgeranylation of RhoA by GGTases is required for its activation by agonists (5). To explore the possibility that insulin may be affecting these two major processes of posttranslational modification to cause Rho inactivation, we examined GGTase-1 activity, the major isoformal present in VSMCs, and examined the effect of the NO/cGMP signaling pathway on GGTase-1 activity. As seen in Fig. 4, thrombin treatment for 10 min resulted in a 1.8-fold increase in GGTase-1. Preexposure to 10 nmol/l insulin for 10 min abolished the thrombin-induced increase in GGTase-1 activity. Insulin alone did not affect basal levels of GGTase-1 activity. Pretreatment with Rp-cGMP and l-NMMA abolished the inhibitory effect of insulin on GGTase-1 and restored the enzyme activity to values.
comparable to thrombin-stimulated cells (Fig. 4). In contrast, 8-bromo-cGMP mimicked insulin’s effect by blocking thrombin-induced GGTase-1 activity. Prolonged incubations for 24 h, with higher concentrations of insulin to mimic hyperinsulinemia, have been reported to increase GGTase-1 activity (27). Decreased GGTase-1 activity was accompanied by a decrease in geranylgeranylated Rho A in the membrane fraction in insulin and 8-bromo-cGMP-treated cells (Fig. 5). This decrease was prevented by RpcGMP and l-NMMA.

An analyses of in vivo RhoA phosphorylation status indicated that RhoA is considerably phosphorylated in the unstimulated VSMCs (Fig. 6A, lane 1 and Fig. 6B). Insulin caused a further twofold increase in RhoA phosphorylation when present alone or added before thrombin (Fig. 6A, lanes 2 and 4, and Fig. 6B). However, wortmannin, a phosphatidylinositol-3 kinase (PI3-kinase) inhibitor, l-NMMA, and RpcGMP all decreased RhoA phosphorylation in untreated insulin-stimulated and insulin-exposed thrombin-stimulated cells (Fig. 6A, lanes 5–11 and Fig. 6B), whereas 8-bromo-cGMP increased RhoA phosphorylation (Fig. 6A, lane 12 and Fig. 6B). ODQ, a guanyl cyclase inhibitor, abolished RhoA phosphorylation when added before insulin treatment (Fig. 6A, lane 13 and Fig. 6B). RhoA antibody, pretreated with peptide antigen, did not immunoprecipitate [32P]-labeled RhoA, suggesting that the protein phosphorylated is RhoA and not some other protein. Further confirmation of the phosphorylation of RhoA was provided by our ability to immunoprecipitate [32P]-labeled RhoA, suggesting that the protein phosphorylated is RhoA and not some other protein.
RhoA and its relationship to RhoA activation came from studies in which active GTP-bound RhoA was precipitated from \[^{32P}\]-labeled VSMCs using glutathione S-transferase (GST)-Rhotekin-RBD pull-down assay. Only active Rho binds to Rhotekin-RBD. Bound RhoA was eluted and subjected to SDS/PAGE and phosphorylation status was determined by autoradiography. The amount of RhoA retained on GST-Rhotekin was determined by probing membranes with Rho antibody. The phosphorylation status of active RhoA was quantitated by densitometric analysis of phosphorylated RhoA bands and corrected for Rho protein by dividing the \[^{32P}\] signal with protein signal.

**FIG. 6.** Inhibitors of PI3-kinase and NOS/cGMP signaling pathway decrease RhoA phosphorylation. \[^{32P}\]-labeled VSMCs were pretreated with wortmannin (100 nmol/l), L-NMMA, RgcGMP, 8-bromo-cGMP, and ODQ (100 μmol/l) for 30 min followed by treatment with and without insulin (100 nmol/l) for 10 min and then exposed to thrombin (1 unit/ml) for 10 min. Equal amounts of precleared lysate proteins (200 μg) were immunoprecipitated with anti-RhoA antibody followed by SDS/PAGE and autoradiography. A: Representative autoradiogram is shown. B: \[^{32P}\] signal from the autoradiograms was quantitated by densitometry and normalized for variations in immunoprecipitated RhoA by dividing the intensity of \[^{32P}\] signal with that of RhoA protein. Results are the means ± SE of three separate experiments expressed relative to untreated control, which was assigned a value of 1. C: \[^{32P}\]-labeled VSMC lysates (250 μg protein) were incubated with 40 μl of GST-Rhotekin-RBD-agarose for 1 h at 4°C. Beads were sedimented by brief centrifugation and washed with Rho wash buffer containing protease and phosphatase inhibitors. Bound RhoA eluted with Laemmli’s sample buffer followed by SDS/PAGE and Western blot analysis with anti-RhoA antibody (top panel). Phosphorylation status of RBD-bound RhoA was examined by autoradiography (middle panel). To verify equal loading 50 μg of cell lysates were subjected to Western blot analysis with RhoA antibody (bottom panel). A representative autoradiogram is shown. D: \[^{32P}\] signal from the autoradiograms was quantitated by densitometry and normalized for RhoA bound to Rhotekin by dividing the intensity of \[^{32P}\] signal with that of Rho protein. Results are the means ± SE of three separate experiments expressed relative to control, which was assigned a value of 1.

RhoA and its relationship to RhoA activation came from studies in which active GTP-bound RhoA was precipitated from \[^{32P}\]-labeled VSMCs using glutathione S-transferase (GST)-Rhotekin-RBD pull-down assay. Only active Rho binds to Rhotekin-RBD. Bound RhoA was eluted and subjected to SDS/PAGE and phosphorylation status was determined by autoradiography. The amount of RhoA retained on GST-Rhotekin was determined by probing membranes with Rho antibody. The phosphorylation status of active RhoA was quantitated by densitometric analysis of phosphorylated RhoA bands and corrected for Rho protein by dividing the \[^{32P}\] signal with protein signal.
As shown in Fig. 6C, upper panel, a small amount of RhoA was associated with GST-Rhotekin-RBD under basal and insulin-stimulated conditions. Thrombin induced a more than threefold increase in active RhoA, which was 70% less phosphorylated when compared with untreated control (Fig. 6C middle panel, lane 3 and Fig. 6D). Both insulin and 8-bromo-cGMP prevented RhoA activation by thrombin and increased Rho phosphorylation (Fig. 6C, top and middle panels, lanes 4 and 12 versus lane 3 and Fig. 6D). Pretreatment with wortmannin, 1-NMMA, RpcGMPS, and ODQ all prevented the effect of insulin, restored Rho activation by thrombin, and decreased Rho phosphorylation (Fig. 6C, top and middle panels, lanes 5–11 versus lane 4 and Fig. 6D). The above effects were specific because RhoA detection could be prevented by inclusion of peptide antigen during incubation with the antibody (see Fig. 6C, top and middle panels, lanes 13 and 14).

**Insulin-mediated inactivation of Rho signaling is accompanied by inhibition of actin cytoskeleton organization.** We also examined the ability of insulin to inhibit actin stress fiber formation in low-passage VSMCs because the higher passage was recently reported to increase actin stress fiber formation (31). Figure 7 shows VSMCs double stained with FITC-conjugated actin antibody and Texas Red-labeled DNase I to colocalize F-actin and monomeric G-actin, respectively. In untreated VSMCs, a few thin actin filaments were observed (Fig. 7A). Insulin treatment caused a decrease in actin filaments (Fig. 7B) and decreased the ratio of F-actin to G-actin by 30%. In contrast, treatment with thrombin caused formation of dense and organized network of thick and parallel actin stress fibers (Fig. 7C), which was prevented by pretreatment with insulin (Fig. 7D). Similar results were observed when PE was used as an agonist (data not shown). More importantly, preincubation with RpcGMPS and 1-NMMA for 1 h decreased the insulin effect and restored thrombin-mediated actin fiber reorganization (Fig. 7E and F), but the actin fibers were not as dense as compared with thrombin alone. 8-Bromo-cGMP, a cGMP agonist, mimicked insulin’s effect by decreasing thrombin-induced actin stress fiber formation (Fig. 7G), as evidenced by increased Texas-Red-labeled DNase I staining and a reduced ratio of F-actin to G-actin.

**DISCUSSION**

The results presented in this study indicate that insulin negatively regulates Rho signaling by inhibiting thrombin-induced RhoA activation via the NO/cGMP signaling pathway. More importantly, insulin, via NO/cGMP, affects posttranslational modification of RhoA through a combination of an upregulation of Rho phosphorylation, resulting in inhibition of Rho activation and translocation, and a decrease in RhoA geranylgeranylation due to inhibition of GGTTase-1 activity. Insulin inactivation of RhoA via NO/cGMP inhibits Rho kinase activity, decreases MBP phosphorylation, and activates MBP, leading to inhibition of actin cytoskeleton organization, which may contribute to the well-known vasodilator actions of insulin.

Several lines of evidence presented in this study suggest that inactivation of RhoA by insulin is mediated by multiple inputs from the NO/cGMP signaling pathway. First, treatment with 1-NMMA, a NOS inhibitor, and Rp-8-bromo-cGMPS, a cGMP antagonist, prevented insulin inactivation of RhoA as well as Rho kinase inactivation, whereas 8-bromo-cGMP, a cGMP agonist, mimicked the inhibitory effect of insulin on thrombin-induced Rho activation/translocation and Rho kinase activity. Second, both insulin and 8-bromo-cGMP inhibit the thrombin-induced increase in GGTase-1 activity, which is effectively blocked by 1-NMMA and Rp-8-bromo-cGMPS. Third, wortmannin, a PI3-kinase inhibitor, 1-NMMA, Rp-8-bromo-cGMPS, and ODQ, a guanylylcyclase inhibitor, all block RhoA phosphorylation and restore RhoA activation by thrombin. It should be noted that although wortmannin prevented the insulin-mediated increase in RhoA phosphorylation and MBP activation, it failed to prevent insulin-mediated inactivation of Rho kinase (21). The reasons for this discrepancy are not clear. It is plausible that Rho kinase inhibition by
insulin may be mediated by other unidentified signaling molecules in addition to inactivation of RhoA.

Earlier studies have shown that inhibition of protein geranylgeranylation causes superinduction of inducible NOS (iNOS) by interleukin-1β in VSMCs (32), suggesting that these two pathways regulate the activation status of each other via a complex cross talk. We have also reported that insulin increases iNOS protein expression as well as cGMP generation in VSMCs (21,23,24). Thus, insulin-induced iNOS expression/cGMP generation attenuates RhoA signaling by direct inactivation of Rho by altering the phosphorylation as well as isoprenylation of RhoA. In contrast, chronic activation of RhoA by vasoconstrictors, as well as by disease states such as hypertension and diabetes, may inhibit iNOS/cGMP generation by specifically blocking insulin signaling via the insulin receptor substrate-1/P3-kinase pathway (21,23,24). We and others have recently reported that the P3-kinase pathway mediates NOS activation in both VSMCs and endothelial cells. In the endothelial cells, NOS is activated via phosphorylation by the P3-kinase downstream target, Akt (33).

Several studies have reported that posttranslational modification of RhoA by geranylgeranylation and phosphorylation plays a major role in RhoA activation (13–16). Although prenylation of proteins is considered a stable modification, we have observed that acute insulin treatment inhibits the thrombin-induced increase in GGTase-1 activity and is prevented by L-NMMA and RapGMPs. GGTase-1 is known to be phosphorylated. It is plausible that thrombin may phosphorylate GGTase-1 to cause its activation, and insulin may inhibit GGTase-1 activity by decreasing the phosphorylation via an activated phosphatase, presumably MBP. Due to the nonavailability of GGTase-1 antibody, we did not measure the phosphorylation status of GGTase-1. Further studies are needed to identify the kinase and phosphatase that may mediate thrombin-induced GGTase-1 phosphorylation and dephosphorylation. Alternatively, insulin may detach the geranylgeranylated moeity from RhoA by proteolysis.

In summary, we have demonstrated that insulin inactivates Rho and prevents Rho signaling by affecting post-translational modification of RhoA via the NO/cGMP pathway to cause Rho kinase inhibition, resulting in a decrease in MBSThr695 phosphorylation, which leads to MBP activation and actin cytoskeleton disorganization.

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INSULIN CAUSES ACTIN DISORGANIZATION VIA Rho INACTIVATION