The mechanism by which 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors increase endothelial nitric oxide synthase expression is unknown. To determine whether changes in isoprenoid synthesis affects eNOS expression, human endothelial cells were treated with the HMG-CoA reductase inhibitor, mevas-tatin (1–10 μM), in the presence of l-mevalonate (200 μM), geranylgeranylpolphosphate (GGPP, 1–10 μM), farnesylpyrophosphate (FPP, 5–10 μM), or low density lipoprotein (LDL, 1 mg/ml). Mevastatin increased eNOS mRNA and protein levels by 305 ± 15% and 180 ± 11%, respectively. Co-treatment with l-mevalonate or GGPP, but not FPP or LDL, reversed mevastatin’s effects. Because Rho GTPases undergo geranylgeranyl modification, we investigated whether Rho regulates eNOS expression. Immunoblot analyses and [35S]GTPγS-binding assays revealed that mevastatin inhibited Rho membrane translocation and GTP binding activity by 60 ± 5% and 78 ± 6%, both of which were reversed by co-treatment with GGPP but not FPP. Farnesyltransferase inhibition of Rho by Clostridium botulinum C3 transferase (50 μg/ml) or by overexpression of a dominant-negative N19RhoA mutant increased eNOS expression. In contrast, activation of Rho by Escherichia coli cytotoxic necrotizing factor-1 (200 ng/ml) decreased eNOS expression. These findings indicate that Rho negatively regulates eNOS expression and that HMG-CoA reductase inhibitors up-regulate eNOS expression by blocking Rho geranylgeranylation, which is necessary for its membrane-associated activity.

Recent large clinical trials have demonstrated that 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors decrease the incidence of ischemic strokes and myocardial infarctions in atherosclerotic and hypercholesterolemic individuals (1, 2). Although the beneficial effects of HMG-CoA reductase inhibitors are primarily attributed to their lipid-lowering effects, subgroup analyses of the data from these trials suggest that there may be beneficial effects of these agents that are independent of serum cholesterol levels (3). For example, the restoration of endothelial function is one of the earliest recognizable benefits after treatment with these agents, sometimes occurring before significant reduction in serum cholesterol levels (4–6). Indeed, we have previously shown that the HMG-CoA reductase inhibitors, simvastatin and lovastatin, reverse the down-regulation of endothelial nitric oxide synthase (eNOS) expression by hypoxia and oxidized low density lipoprotein (LDL) under cholesterol-clamped conditions (7, 8). Although hypercholesterolemia impairs endothelial function and plasma LDL apheresis improves endothelium-dependent vasodilation (9–11), there is growing evidence that HMG-CoA reductase inhibitors may have additional effects on eNOS activity beyond that of cholesterol reduction.

By inhibiting l-mevalonate synthesis, HMG-CoA reductase inhibitors also prevent the synthesis of other important isoprenoid intermediates of the cholesterol biosynthetic pathway, such as farnesylpyrophosphate (FPP) and geranylgeranylpolphosphate (GGPP) (12). The isoprenoids are important lipid attachments for the post-translational modification of variety of proteins, including the γ subunit of heterotrimeric G proteins, heme a, nuclear lamins, Ras, and Ras-like proteins, such as Rho, Rab, Rac, Ral, or Rap (13, 14). These isoprenoid intermediates permit the covalent attachment, subcellular localization, and intracellular trafficking of membrane-associated proteins. The role that isoprenoids play in regulating eNOS expression, however, is not known. The purpose of this study, therefore, is to identify the isoprenoid intermediate(s) and the post-translationally modified protein(s) which regulate eNOS expression.

EXPERIMENTAL PROCEDURES

Materials—All standard culture reagents were obtained from JRH Bioscience (Lenexa, KS). Mevastatin, farnesylpyrophosphate, geranylgeranylpolphosphate, l-mevalonate, and 5,6-dichlorobenzimidazo-lole riboside (DRB) were purchased from Sigma. Mevastatin (compari-son) was chemically activated by alkaline hydrolysis prior to use as described previously (7). FPT inhibitor I and α-hydroxyfarnesylphosphonic acid were purchased from Calbiochem. [α-32P]CTP (3000 Ci/mmoll) and [35S]GTPγS (1250 Ci/mmoll) were supplied by NEN Life Science Products. The antibody detection kit (Enhanced Chemiluminescence) and the nylon nucleic acid (Hybond) and protein (polyvinylidene difluoride) transfer membranes were purchased from Amersham Corp. The Clostridium botulinum C3 transferase was purchased from List Biological Laboratories, Inc. (Campbell, CA). Recombinant Escherichia coli cytotoxic necrotizing factor (CNF)-1, RhoA mutants, and [α-1.6 kb] eNOS-luciferase promoter construct were kindly provided by K. Akto-ries (University of Freiberg, Freiberg, Germany), W. Moolenaar (Neth-erlands Cancer Institute, Amsterdam, The Netherlands), and W. Sessa (Yale University, New Haven, CT), respectively.

Cell Culture—Human endothelial cells were harvested using Type II collagenase (Worthington) and cultured in Medium 199, 20 mE HEPES,
50 μg/ml endothelial cell growth serum (Collaborative Research Inc., Bedford, MA), 100 μg/ml heparin sulfate, 5 mm l-glutamine (Life Technologies, Inc.), 5% fetal calf serum (HyClone, Logan, UT), and antibiotic mixture of penicillin (100 units/ml)/streptomycin (100 μg/ml)/fungizone (1.25 μg/ml) as described previously (7–10). For transfection studies, bovine aortic endothelial cells of less than three passages were cultured in a growth medium containing Dulbecco's modified Eagle's medium, 5 mm l-glutamine (Life Technologies, Inc.), and 10% fetal calf serum. Cellular viability was determined by cell count, morphology, and trypan blue exclusion.

**Preparation of LDL**—The LDL was prepared by discontinuous ultra-centrifugation of freshly isolated plasma according to the method of Chung et al. (15). The purity of the LDL samples was confirmed by SDS-polyacrylamide and cellulose acetate gel electrophoresis. Protein, cholesterol, and triglyceride content were determined as described previously (8–10). For comparison, commercially available LDL (Biomedical Technologies Inc., Stoughton, MA; Calbiochem, San Diego, CA) were similarly characterized and used in selected experiments. The extent of LDL oxidation was estimated by assaying for thiobarbituric acid-reactive substances and expressed as nanomoles of malondialdehyde per mg of LDL protein (16). Only freshly isolated LDL with thiobarbituric acid-reactive substance values of less than 0.5 mmol/mg was used in this study.

**Northern Blotting**—Equal amounts of total RNA (20 μg) were separated on 1.5% formaldehyde-agarose gel electrophoresis and blotted (using 10 μg/ml, Bio-Rad) onto nylon membranes. The RNA loading was determined by ethidium bromide staining of 18 S and 28 S ribosomal RNA on the nylon membranes.

**Western Blotting**—Proteins were prepared and separated on SDS-PAGE as described (7–10). Immunoblotting was performed using monoclonal antibodies to eNOS (1:2000 dilution, Transduction Laboratories, Lexington, KY), to RhoA and RhoB (1:250 dilution, Santa Cruz Biotechnology Inc., Santa Cruz, CA), and to c-myc tag (9E10, 1:200 dilution, Santa Cruz Biotechnology Inc.). Immunodetection was accomplished using a sheep anti-mouse secondary antibody (1:4000 dilution) or donkey anti-rabbit secondary antibody (1:4000 dilution) and the enhanced chemiluminescence (ECL) kit (Amersham Corp.). Autoradiography was performed at 23 °C, and the appropriate exposures were quantitated by densitometry.

**Assay for Rho GTP Binding Activity**—The Rho GTP binding activity was determined by immunoprecipitation of [32P]GTP-S-labeled Rho. Briefly, membrane and cytosolic proteins were isolated as described previously (10, 17). The [32P]GTP-S binding assay was performed as described (10). Samples were then reconstituted in 100 μl of immunoprecipitation buffer containing Triton X-100 (1%), SDS (0.1%), NaCl (150 mM), EDTA (5 mM), Tris-HCl (25 mM, pH 7.4), leupeptin (10 μg/ml), aprotinin (10 μg/ml), and phenylmethylsulfonyl fluoride (2 mM). The RhoA or RhoB antisera were added to the mixture at a final dilution of 1:75. The samples were allowed to incubate for 16 h at 4 °C with gentle mixing. The antibody-activated protein complexes were then incubated with 50 μl of protein A-Sepharose (1 mg/ml, Amersham Pharmacia Biotech) for 2 h at 4 °C, and the immunoprecipitate was collected by centrifugation at 12,000 × g for 10 min.

Preliminary studies using Western analysis of the supernatant indicated that both RhoA and RhoB were completely immunoprecipitated under these conditions. The pellets were washed four times in a buffer containing HEPES (50 mM, pH 7.4), NaF (100 mM), sodium phosphate (50 mM), NaCl (100 mM), Triton X-100 (1%), and SDS (0.1%). The final pellet containing the immunoprecipitated [32P]GTP-S-labeled Rho proteins was counted in a liquid scintillation counter (LS 1800, Beckman Instruments). Nonspecific activity was determined in the presence of excess unlabeled GTP-S (100 μM).

**Overexpression of Rho Mutants**—For transfection studies, bovine rather than human endothelial cells were used because of their higher transfection efficiency by the calcium phosphate precipitation method (8). Bovine endothelial cells (60–70% confluent) were transfected with 15 μg of the indicated cDNAs: the insertless vector (pcDNA3), pcDNA3-c-myc-wtRhoA (wild type RhoA), pcDNA3-c-myc-RhoA (dominant-negative RhoA mutant), or a [-1.6 kb] eNOS promoter linked to the luciferase reporter gene (18). As an internal control for transfection efficiency, pcCMV-β-galactosidase (15 μg) was co-transfected. Preliminary results using β-galactosidase staining indicate that cellular transfection efficiency was approximately 14%.

Approximately 24 h after transfection, cells were harvested for immunoblots analyses of eNOS expression. The eNOS protein levels were then standardized to the corresponding levels of transfected RhoA expression as determined by antisera to the corresponding c-myc tag. For luciferase activity, cells were harvested 48 h after transfection. The β-galactosidase and luciferase activities were determined by a chemiluminescence assay (Dual-Light, Tropix, Bedford, MA) using a Berthold LB9501 luminometer. The eNOS promoter activity was expressed as the ratio of luciferase to β-galactosidase activity.

**Results**—The eNOS activity was determined by a modified nitrite assay using 2,3-diaminonaphthalene as described previously (7). Fluorescence of 1-H-naphthotriazole was measured with excitation and emission wavelengths of 365 and 450 nm, respectively. Standard curves were constructed with known amounts of sodium nitrite. Nonspecific fluorescence was determined in the presence of LNMA (3 μM).

**Cell Culture**—Relatively pure (>98%) human saphenous vein and aortic endothelial cell cultures were confirmed by their morphological features (i.e. cuboidal, cobblestone, contact-inhibited) using phase-contrast microscopy and immunofluorescent staining with antibodies to Factor VIII (data not shown). To determine which downstream isoprenoid intermediate in the cholesterol bio-synthetic pathway regulates eNOS expression, endothelial cells were treated with mevalonate (10 μM) in the presence of isoprenoid intermediates, geranylgeranylpyrophosphate (GGPP), farnesylpyrophosphate (FPP), or LDL-cholesterol. Co-treatment with FPP (10 μM) or LDL (1 mg/ml) did not significantly reverse the effects of mevalonate on eNOS protein levels. Furthermore, inhibition of protein farnesyltransferase with the FTase inhibitor I (0.5–50 μM) or α-hydroxyfarnesylphosphonic acid (2–20 μM) did not affect eNOS protein levels (data not shown). In contrast, co-treatment with GGPP at a concentration of 10 μM, but not 1 μM, completely reversed the up-regulation of eNOS protein levels by mevalonate (Fig. 1). These findings indicate that eNOS expression is negatively regulated by geranylgeranyl synthesis. Similar effects were observed in aortic endothelial cells and with the more lipophilic isoprenoid derivatives, geranylgeraniol and farnesol (data not shown).

**Effects of Isoprenoids on eNOS mRNA Expression**—We previously reported that inhibition of endothelial HMG-CoA reductase by lovastatin or simvastatin up-regulates eNOS expression and activity (7, 8). Similarly, treatment with mevalonate (10 μM) increased eNOS protein levels by 180 ± 11% after 24 h (p < 0.05, n = 4) (Fig. 1). To determine which downstream isoprenoid intermediate in the cholesterol bio-synthetic pathway regulates eNOS expression, endothelial cells were treated with mevalonate (10 μM) in the presence of isoprenoid intermediates, geranylgeranylpyrophosphate (GGPP), farnesylpyrophosphate (FPP), or LDL-cholesterol. Co-treatment with FPP (10 μM) or LDL (1 mg/ml) did not significantly reverse the effects of mevalonate on eNOS protein levels. Furthermore, inhibition of protein farnesyltransferase with the FTase inhibitor I (0.5–50 μM) or α-hydroxyfarnesylphosphonic acid (2–20 μM) did not affect eNOS protein levels (data not shown). In contrast, co-treatment with GGPP at a concentration of 10 μM, but not 1 μM, completely reversed the up-regulation of eNOS protein levels by mevalonate (Fig. 1). These findings indicate that eNOS expression is negatively regulated by geranylgeranyl synthesis. Similar effects were observed in aortic endothelial cells and with the more lipophilic isoprenoid derivatives, geranylgeraniol and farnesol (data not shown).

**Effects of Isoprenoids on eNOS mRNA Expression**—Treatment of endothelial cells with mevalonate (10 μM) increased eNOS steady-state mRNA levels by 305 ± 15% after 24 h (Fig. 2A). On a molar basis, we find that mevalonate is equally
Geranylgeranylation of Rho Decreases eNOS Expression

Effects of Mevastatin on Rho Membrane Translocation—The geranylgeranylation of the small GTPases, RhoA and RhoB, are essential for their membrane translocation from the cytosol (20). Under basal culture conditions, both RhoA and RhoB are present in the membranes and cytosol (Fig. 3). Treatment with mevastatin decreased membrane localization of RhoA and RhoB by 60 ± 5% and 78 ± 6% with reciprocal concomitant increases in RhoA and RhoB in the cytosol by 65 ± 4% and 87 ± 7% (p < 0.05 compared with basal condition for all values). Co-treatment with GGPP (5 µM), but not FPP (10 µM), reversed the effects of mevastatin and completely restored the amount of cytosolic and membrane-associated RhoA and RhoB to basal levels.

Mevastatin also decreased membrane-associated Ras by 55 ± 7% with concomitant appearance of a higher molecular weight Ras in the cytosolic extracts (Fig. 3). Previous studies have shown that farnesylated Ras migrates slightly faster on SDS-PAGE than unmodified Ras (21). In contrast to RhoA and RhoB, membrane-associated Ras was completely restored in the presence of FPP, but not GGPP. These findings suggest that inhibition of Rho geranylgeranylation by mevastatin specifically prevents RhoA and RhoB from translocating to and associating with the cellular membrane.

Effects of Mevastatin on Rho GTP Binding Activity—To determine whether geranylgeranylation of RhoA and RhoB affects their activity (i.e. GTP-bound state), we immunoprecipitated [35S]GTPγS-labeled RhoA and RhoB from the membrane and cytosol of endothelial cells treated with mevastatin (10 µM) in the presence of GGPP (5 µM) or FPP (10 µM) (Fig. 4). Under basal conditions, endothelial cells have membrane-associated RhoA and RhoB activity of 4.4 ± 0.1 fmol/mg/min and 3.8 ± 0.4 fmol/mg/min, respectively. Treatment with mevastatin decreased membrane-associated RhoA and RhoB GTP binding activity by 52% (2.1 ± 0.4 fmol/mg/min; p < 0.01, n = 3) and 37% (2.4 ± 0.6 fmol/mg/min; p < 0.05, n = 3), respectively.

Co-treatment with FPP (10 µM) produced no significant effects on RhoA and RhoB GTP binding activity compared with mevastatin alone (2.6 ± 0.9 fmol/mg/min and 2.7 ± 0.5 fmol/mg/min, respectively, p > 0.05, n = 3) (Fig. 5). However, co-treatment with GGPP (10 µM) completely reversed the inhibitory effects of mevastatin on RhoA and RhoB GTP binding activity (4.1 ± 0.3 fmol/mg/min and 3.6 ± 0.5 fmol/mg/min, respectively, p < 0.05, n = 3). Cytosolic RhoA and RhoB are relatively inactive in the GDP-bound state, and therefore, their GTP binding activities (i.e. <1 fmol/mg/min) were not signifi-

Fig. 3. Immunoblots (30 µg of protein/lane) showing the effects of mevastatin (Statin, 10 µM) alone or in combination with FPP (10 µM) or GGPP (5 µM) on cytosolic and membrane-associated RhoA, RhoB, and Ras protein levels after 24 h. Each blot is representative of four separate experiments.
Geranylgeranylation of Rho Decreases eNOS Expression

**Fig. 4.** The effects of mevastatin (Statin, 10 μM) alone or in combination with FPP (10 μM) or GGPP (5 μM) on cytosolic and membrane-associated RhoA (A) and RhoB (B) GTP binding activity after 24 h. *, p < 0.05; **, p < 0.01 compared with control (untreated). All experiments were performed at least three times in duplicate.

significantly affected by treatment with mevastatin alone or in combination with GGPP or FPP (p > 0.05).

**Effects of Rho Modulators on eNOS Expression**—To determine whether the inhibition of Rho mediates the effects of mevastatin on eNOS expression, endothelial cells were treated with mevastatin in the presence and absence of *C. botulinum* C3 transferase (5–50 μg/ml), an exoenzyme which inactivates Rho by ADP-ribosylation (22). Treatment of endothelial cells with mevastatin (10 μM) or C3 transferase (50 μg/ml) for 48 h augmented eNOS protein levels by 260 ± 9% and 250 ± 10%, respectively (p < 0.01, n = 3) (Fig. 5A). Lower concentrations of C3 transferase (i.e. <50 μg/ml) produced correspondingly smaller increases in eNOS expression (data not shown). In contrast to the effect of mevastatin, the stimulatory effect of C3 transferase on eNOS expression, however, was not reversed in the presence of l-mevalonate (200 μM).

Overexpression of wtRhoA slightly decreased basal eNOS protein expression by 20% suggesting that endogenous RhoA activity exerts near-maximal inhibition on eNOS expression under basal conditions (Fig. 5B). In contrast, endothelial cells overexpressing N19RhoA mutant to comparable levels as wtRhoA as assessed by the amount of c-myc tag exhibited a 150 ± 5% increase in eNOS protein levels (p < 0.05, n = 3). Immunostaining for β-galactosidase activity demonstrates comparable transfection efficiency among the RhoA constructs and between treatment conditions. These findings are consistent with our earlier findings that inhibition of Rho GTPase activity by HMG-CoA reductase inhibitors or C3 transferase leads to an increase in eNOS expression.

Previous nuclear run-on assays and eNOS promoter studies did not show any effect of HMG-CoA reductase inhibitors on eNOS gene transcription (7, 8). To determine whether Rho affects eNOS promoter activity, endothelial cells were transfected with a functional [−1.6 kb] eNOS promoter (C). Cells were exposed to laminar flow (12 dynes/cm², 24 h), co-transfected with wild type (wt) or dominant-negative Rho mutant (N19), or treated with C3 transferase (50 μg/ml). The promoter activity for each condition was standardized by β-galactosidase activity and expressed relative to the basal transcriptional activity of C (fold induction). The * represented a significant change in promoter activity compared with C (p < 0.05).

**Fig. 5.** A, immunoblot (30 μg of protein/lane) showing the effects of C3 transferase (C3, 50 μg/ml), mevastatin (Statin, 10 μM), or l-mevalonate (Mev, 200 μM) on eNOS protein levels after 48 h. The blot is representative of three separate experiments. B, immunoblot (30 μg of protein/lane) showing eNOS protein levels after transfection with insertless vector, pCDNA3 (c), c-myc-wild type-RhoA (wt), and c-myc-N19RhoA (dominant-negative rhoA mutant). The levels of overexpressed RhoA mutants were determined by immunoblotting for their corresponding c-myc-tags (c-myc-RhoA). Experiments were performed three times with similar results. C, eNOS promoter activity was assessed by luciferase assays on bovine aortic endothelial cells transfected with plasmid vectors containing no promoter (vector) or the [−1.6 kb] eNOS promoter (C). Cells were exposed to laminar flow (12 dynes/cm², 24 h), co-transfected with wild type (wt) or dominant-negative Rho mutant (N19), or treated with C3 transferase (50 μg/ml). The promoter activity for each condition was standardized by β-galactosidase activity and expressed relative to the basal transcriptional activity of C (fold induction). The * represented a significant change in promoter activity compared with C (p < 0.05).
Geranylgeranylation of Rho Decreases eNOS Expression

In this study, we have identified the isoprenoid intermediate, geranylgeraniol, and its post-translationally modified protein, Rho, as important regulators of eNOS expression. Treatment with HMG-CoA reductase inhibitor, mevastatin, decreases Rho geranylgeranylation, membrane translocation, and GTP binding activity and leads to the up-regulation of eNOS expression. All of these effects of mevastatin were reversed in the presence of GGPP, but not FPP or LDL cholesterol, indicating that geranylgeranyl modification of Rho negatively regulates eNOS expression. Interestingly, treatment with l-mevalonate or GGPP alone did not affect eNOS expression suggesting maximal Rho geranylgeranylation under basal tissue culture conditions. However, direct activation of Rho by GGPP (5 μM) resulted in a decrease in basal eNOS expression suggesting that further activation of membrane-associated Rho or perhaps activation of cytosolic Rho leads to decreases in eNOS expression below basal levels.

The Rho GTPase family, which includes RhoA, RhoB, Rac, and Cdc42, are major substrates for post-translational modification by geranylgeranylation and that geranylgeranylation targets these Rho GTPases to the membrane (12, 13, 20). The membrane translocation of inactive or GDP-bound Rho involves the release of its cytoplasmic inhibitor, Rho guanine nucleotide dissociation inhibitor, and activation of Rho through GDP/GTP exchange in the presence of guanine nucleotide exchange factor (25, 26). Because most of the cytoplasmic Rho proteins are inactive (GDP-bound state), their GTP binding activity remains invariably low even with statin treatment. Thus, inhibition of Rho geranylgeranylation and membrane translocation by mevastatin leads to a greater accumulation of inactive Rho in the cytoplasm. This is consistent with our finding that addition of GGPP, but not FPP, restores membrane expression and activity of Rho and results in the down-regulation of eNOS expression.

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The inhibitory effect of Rho on eNOS expression was further confirmed by studies showing that inhibition of Rho by C. botulinum C3 transferase also increases eNOS expression. The C3 transferase ADP-ribosylates asparagine 41 of RhoA and RhoB and renders them biologically inactive in the GDP-bound state (22). Although under certain circumstances the C3 transferase may also ADP-ribosylate Rac-1 or Cdc42 (22, 24), our results showing that the overexpression of a dominant-negative RhoA mutant, N19RhoA, increases eNOS expression are consistent with the inhibitory effects of C3 transferase on Rho. In contrast, direct activation of Rho by E. coli CNF-1 leads to a decrease in eNOS expression (24). These results, therefore, identify membrane-associated Rho as a negative regulator of eNOS expression and activity.

Previous actinomycin D studies indicate that HMG-CoA reductase inhibitors prolong eNOS mRNA half-life from approximately 30 to 45 h (7, 8). However, such relatively small increases in eNOS mRNA stability cannot entirely account for the 3-fold increase in steady-state eNOS mRNA levels after 24 h. Thus, it is possible that the actinomycin D used in our previous studies may not be the optimal reagent to assess eNOS mRNA stability because it may produce false prolongation of eNOS mRNA half-life by either interacting directly with eNOS mRNA or by preferentially inhibiting the transcription and subsequent translation of protein(s) which degrades eNOS mRNA. Because we have previously established that HMG-CoA reductase inhibitors have no effect on eNOS gene transcription (7, 8), our finding showing that mevastatin increased eNOS mRNA half-life from 14 to 27 h with another RNA polymerase inhibitor, DRB, is in closer agreement with the expected
Geranylgeranylation of Rho Decreases eNOS Expression

increase in steady-state eNOS mRNA levels after 24 h. The activation of Rho leads to actin stress-fiber formation, focal adhesion assembly, and reorganization of the actin cytoskeleton (20, 27). Because HMG-CoA reductase inhibitors up-regulate eNOS expression predominantly via stabilization of eNOS mRNA, it is likely from the findings of this study that Rho-mediated cytoskeletal changes affect eNOS mRNA stability. Recent studies demonstrate that cyttoplasmatic filaments and microtubules are necessary to transport mRNAs within the cytoplasm and to anchor them at specific subcellular locations (28, 29). The anchoring of mRNAs to the cytoskeleton and their co-localization with ribosomes and RNA-binding protein complexes are necessary for their translational expression and stability. Thus, Rho-controlled reorganization of the actin cytoskeleton may play a key role in the movement and compartmentalization of specific mRNAs. For example, the peripheral localization of β-actin mRNA is required for the assembly of contractile and motility apparatus at the leading edge of moving cells (29). Thus, it is possible that Rho decreases eNOS mRNA translation and stability via effects on the cytoskeletal localization of eNOS mRNA. Endothelial dysfunction, which is often defined as a decrease in eNOS activity or NO bioavailability is one of the earliest manifestations of atherosclerosis, occurring even in the absence of angiographic evidence of disease (30–32). Atherogenic risk factors and decreased eNOS activity are strongly correlated, and improved endothelial function is an early clinical marker following atherogenic risk factor modification. For example, LDL, especially oxidized LDL, is a potent inhibitor of endothelial function (9, 33). The mechanisms by which oxidized LDL inhibits endothelial function include down-regulation of eNOS activity (9), decreased receptor-mediated NO release (10, 34), and NO inactivation via increases in superoxide anion production (35). Indeed, endothelial dysfunction is a hallmark of hypercholesterolemia and rapidly improves with cholesterol reduction (4, 5, 11). However, some of the beneficial effects of HMG-CoA reductase inhibitors in terms of eNOS expression and restoration of endothelial function occur via mechanisms independent of serum cholesterol levels (3, 6–8).

In summary, we have shown that the up-regulation of eNOS expression by HMG-CoA reductase inhibitors is mediated by inhibition of Rho GTPase. Because decreases in eNOS expression and activity may contribute to the pathogenesis of atherosclerotic coronary artery disease and pulmonary hypertension, therapeutic modalities used to selectively inhibit endothelial Rho activity may prove to be beneficial. We propose that some of the beneficial effects of HMG-CoA reductase inhibitors in cardiovascular diseases are not related to their cholesterol-lowering effects but to their ability to inhibit Rho geranylgeranylation. The mechanism(s) by which Rho decreases the stability of eNOS mRNA, however, remains to be determined.

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