Inhibition of geranylgeranylation blocks agonist-induced actin reorganization in human airway smooth muscle cells

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Inhibition of geranylgeranylation blocks agonist-induced actin reorganization in human airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 281: L824–L831, 2001.—To determine whether RhoA isoprenylation (geranylgeranylation) is required for agonist-induced actin cytoskeleton reorganization (measured by an increase in the filamentous F- to monomeric G-actin ratio), human airway smooth muscle cells were treated for 72 h with inhibitors of geranylgeranyltransferase I. Geranylgeranyltransferase inhibitor (GGTI)-2147 or -286 pretreatment completely blocked the increase in the F- to G-actin fluorescence ratio when cells were stimulated with lysophosphatidic acid (LPA), endothelin, or carbachol. In contrast, LPA or endothelin induced actin cytoskeletal reorganization in cells treated with farnesyltransferase inhibitor (FTI)-277 to inactivate Ras. Forskolin-induced adenyl cyclase activity was inhibited by carbachol in GGTI-2147-pretreated cells, demonstrating that the effect of geranylgeranyltransferase I inhibition on stress fiber formation was not due to uncoupling of signaling between the heterotrimeric G protein (the G\textsubscript{i} subunit is isoprenylated) and distal effectors. These results demonstrate that selective GGTIs can inhibit agonist-induced actin reorganization.

fluorescence microscopy; prenylation; prenyltransferase inhibition; cytoskeleton; actin depolymerization

THE MONOMERIC G PROTEIN RhoA plays an important role in actin cytoskeletal organization, cell proliferation, and signal transduction (38, 44). Pretreatment of cells with Clostridium botulinum exotoxin, C3, inactivates RhoA and inhibits agonist-induced actin reorganization in many cell types, including human airway smooth muscle cells (15, 17, 43). Microinjection of either a constitutively active RhoA mutant (Val\textsuperscript{14} RhoA) (33) or Rho kinase, a distal effector of activated RhoA (2), also induces actin reorganization. These data underscore the importance of RhoA in actin cytoskeletal rearrangements. Recent work in an experimental model of asthma has shown that RhoA expression is increased in the smooth muscle cells of animals that are sensitized to allergens (9), which has also stimulated our interest in the role of RhoA in airway smooth muscle cells.

In quiescent cells, RhoA is found mainly in the cytosol, associated with its guanine nucleotide dissociation inhibitor (GDI). Dissociation from the GDI and translocation to the cell membrane appear necessary for some effector interactions of RhoA, including agonist-induced calcium sensitization in smooth muscle. Inactivation of RhoA with a chimeric C\textsubscript{3} exotoxin inactivates RhoA, interrupts RhoA membrane translocation, and inhibits activation of agonist-induced calcium sensitization (14). It is not known, however, whether RhoA must translocate to the membrane to induce agonist-induced reorganization of the actin cytoskeleton in human airway smooth muscle cells.

To interact with the plasma membrane or with its associated regulatory proteins [guanine nucleotide exchange factor (GEF) or GDI], RhoA, like Ras, must be posttranslationally isoprenylated at the carboxy (COOH) terminus (5, 7, 19, 36). The posttranslational processing includes COOH-terminal isoprenylation ([\textsuperscript{20}C]geranylgeranylation for Rho and [\textsuperscript{15}F]farnesylation for Ras), cleavage of the three COOH-terminal amino acids, and COOH-terminal methylation (21). Transfer of geranylgeranyl pyrophosphate to the COOH terminus of RhoA is catalyzed by the zinc metalloenzyme geranylgeranyltransferase I (40). Inhibitors of geranylgeranyltransferase I (46) block posttranslational geranylgeranylation and would be predicted to inhibit the ability of RhoA to translocate from the cytosol to the plasma membrane; this has previously been shown in other cell types (25, 31).

It is not known whether RhoA must undergo plasma membrane translocation to mediate agonist-induced actin reorganization in human airway smooth muscle cells. If RhoA plays a central role in cytoskeletal reorganization (17, 43) and if translocation is important for RhoA activation of actin cytoskeletal reorganization, then inhibition of RhoA geranylgeranylation should inhibit agonist-induced actin reorganization in these...
cells. Inhibition of prenylation is sufficient to inhibit membrane association of RhoA on activation (1). To test this hypothesis, we pretreated human airway smooth muscle cells [previously shown to express receptors that respond to lysophosphatidic acid (LPA), endothelin, and carbachol] with vehicle (DMSO) and one of two geranylgeranyltansferase inhibitors (GGTIs; GGTI-2147 or GGTI-286) for 72 h. To exclude the possibility that Ras proteins play a major role in agonist-induced actin reorganization and that the GGTIs block farnesylation nonspecifically, cells were treated for 72 h with farnesyltransferase inhibitor (FTI)-277. Agonist-induced changes in the fluorescence intensity ratios of filamentous (F) and globular (G) actin were then determined by fluorescence microscopy.

METHODS

Cell culture. Primary cultures of previously characterized human tracheal smooth muscle cells (32) were maintained in Ham’s F-12 medium containing antibiotics (100 units/ml of penicillin G, 100 μg/ml of streptomycin, and 0.25 μg/ml of amphotericin B) and 10% fetal bovine serum at 37°C in an atmosphere of 5% CO₂-95% air. Preliminary immunohistological studies performed in our laboratory confirmed that >90% of cells expressed α-actin. Moreover, immunoblot analysis of these cells identified expression of both α-actin and desmin, confirming the smooth muscle phenotype. Cells were plated on 8-well microscope slides (Nunc, Naperville, IL) and grown until nearly confluent. Cells were used between passages 3 and 5, had undergone approximately nine doublings, and were then treated with either of two GGTIs [GGTI-286 (10 μM) or GGTI-2147 (10 μM)] or FTI-277 (10 μM) in 10% serum for 24 h. These concentrations were previously shown to inhibit geranylgeranyltansferase I [GGTI-2147 (45) and GGTI-286 (28)] or farnesyltransferase (FTI-277) (27) completely in cultured cells. At the end of 24 h, the cells were extensively washed and then maintained in serum-free medium (Ham’s F-12) containing 10 μM of either GGTI-286, GGTI-2147, or FTI-277 for an additional 48 h. The inhibitors were redosed every 24 h. Quiescent, serum-starved cells were maintained in serum-free Ham’s F-12. GGTI-2147 or the DMSO vehicle was added to the medium every 24 h. At the end of 72 h, the medium was aspirated from each well, and the cells were rinsed once with 0.5 ml of warm PBS. Cells were lysed for 60 min at 37°C in 100 μl/well of warm hypotonic lysis buffer (10 mM HEPES, 2 mM EDTA, and 100 μM phenylmethylsulfonyl fluoride, pH 8.0), and cell lysates were immediately assayed for adenyl cyclase activity in the wells in a final volume of 150 μl. Adenyl cyclase activity was measured for 10 min at 30°C in cyclase buffer (final concentrations [in mM]: 0.5 3-isobutyl-1-methylxanthine, 50 HEPES (pH 8.0), 50 NaCl, 0.4 EGTA, 1 cAMP, 7 MgCl₂, and 1 ATP as well as 0.25 mg/ml BSA and 7–20 μCi/ml [α-32P]ATP) and 10 μM forskolin or 10 μM forskolin plus 1 μM carbachol. Adenyl cyclase activity was quantified by measuring the synthesis of [32P]cAMP from [α-32P]ATP. [32P]cAMP was recovered by sequential column chromatography as previously described (11).

Materials. LPA and FITC-phalloidin were obtained from Sigma (St. Louis, MO). Texas Red-DNase I was obtained from Molecular Probes (Eugene, OR). GGTI-286, GGTI-2147, and FTI-277 were purchased from Calbiochem (La Jolla, CA). Vectashield H-1000 was obtained from Vector Laboratories.

Statistical analysis of data. To control for daily variations in staining intensity, untreated cells were always compared with treated cells on the same microscope slide, since cells on the same slide were subjected to identical culture, fixation, permeabilization, staining, and microscopy conditions, allowing for meaningful comparisons among samples. All data are presented as means ± SE. F- to G-actin ratios were compared by two-way analysis of variance with a Bonferroni post hoc comparison test, using Instat software (Graph Pad, San Diego, CA). P < 0.05 was considered significant.
RESULTS

Exposure of serum-deprived human airway smooth muscle cells to 1 µM LPA, 1 µM endothelin, or 10 µM carbachol for 5 min increased the FITC-phalloidin fluorescence intensity of F-actin and decreased the Texas Red-DNase I fluorescence of G-actin (indicating the reorganization of G-actin into F-actin fibers) (Figs. 1 and 2). The F- to G-actin fluorescence ratio increased from 1.5 ± 0.09 in untreated (control) cells and from 1.4 ± 0.10 in the DMSO-treated (vehicle) cells (P > 0.05) to 3.0 ± 0.23 in the LPA-treated cells (P < 0.001; n = 6 experiments) (Fig. 3). Endothelin increased the ratio of F- to G-actin fluorescence from 1.6 ± 0.18 in the untreated (control) cells and 1.5 ± 0.23 in the vehicle (DMSO)-treated cells (P > 0.05) to 2.5 ± 0.41 (P < 0.001) (Fig. 4). Carbachol increased the F- to G-actin fluorescence ratio from a baseline value of 2.3 ± 0.39 in control cells to 3.5 ± 0.55 (P < 0.05; n = 4) (Fig. 5). GGTI-2147 completely blocked the carbachol-induced increase in F- to G-actin fluorescence. Pretreatment of human airway smooth muscle cells with either of two GGTIs (GGTI-286 or GGTI-2147) for 72 h (to prevent RhoA isoprenylation) significantly decreased basal actin reorganization and completely inhibited actin reorganization induced by either LPA, endothelin, or carbachol. In the absence of agonist, GGTI-2147 pretreatment itself significantly decreased the F- to G-actin fluorescent staining ratio from 1.4 ± 0.10 to 0.7 ± 0.05 (P < 0.05). Moreover, in GGTI-2147-pretreated cells, LPA increased the F- to G-actin fluorescence ratio to only 1.0 ± 0.16, which was not significantly different from the F- to G-actin fluorescence ratio in the absence of LPA (P > 0.05) but was significantly different from the F- to G-actin fluorescence ratio seen in cells exposed to LPA in absence of GGTI pretreatment (P < 0.001; n = 6 experiments) (Fig. 3). The F- to G-actin fluorescence ratio was 0.68 ± 0.10 in GGTI-2147-pretreated cells not exposed to endothelin and 0.88 ± 0.03 in the GGTI-2147-pretreated cells exposed to endothelin (P < 0.05; n = 4) (Fig. 4). Similarly, F- to G-actin fluorescence ratios were 0.99 ± 0.11 in control cells and 0.94 ± 0.09 in cells pretreated with GGTI-2147 and subsequently stimulated with carbachol (P > 0.05; n = 4). In cells treated separately with GGTI-286, the F- to G-actin fluorescence ratio was 0.85 ± 0.07 in the absence of agonist and 0.70 ± 0.14 and 0.8 ± 0.01 in LPA- and endothelin-treated cells, respectively. These data suggest that isoprenylation (and, therefore, translocation) of RhoA to the plasma membrane is necessary for both basal and agonist-induced actin reorganization.

In separate experiments, pretreatment of airway smooth muscle cells with the FTI (FTI-277) for 72 h (to inhibit Ras isoprenylation) had no effect on either

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**Fig. 1.** A–C: fluorescence photomicrographs of F-actin-stained human airway smooth muscle cells pretreated with vehicle (DMSO). D–F: F-actin-stained cells pretreated with geranylgeranyltransferase inhibitor (GGTI)-2147. Cells pretreated with DMSO show increased F-actin staining after treatment with either lysophosphatidic acid (LPA; B) or endothelin (C), whereas cells pretreated with GGTI-2147 show no increase in F-actin staining with LPA (E) or endothelin (F) treatment.

**Fig. 2.** A–C: fluorescence photomicrographs of F-actin-stained cells that have been pretreated with vehicle (DMSO) and stimulated with no agonist (A), LPA (B), or endothelin (C). D–F: cells pretreated with the farnesyltransferase inhibitor (FTI)-277 and no agonist (D), LPA (E), or endothelin (F). LPA and endothelin increase the F- to G-actin ratio both in cells that have and in cells that have not been pretreated with FTI-277 (B, C, E, and F).
basal or LPA-induced actin reorganization. The F- to G-actin fluorescence ratio averaged 1.2 ± 0.10, 1.3 ± 0.14, and 1.1 ± 0.11 in untreated, DMSO-treated, and FTI-277-treated cells, respectively, in the absence of LPA (P > 0.05) (Fig. 6). LPA significantly increased the F- to G-actin fluorescence ratio to 2.6 ± 0.26 in FTI-277-pretreated cells (P < 0.001 for LPA compared with each control; n = 6 experiments) (Fig. 6). Endothelin also significantly increased the F- to G-actin fluorescence ratio to 2.3 ± 0.17 and 1.7 ± 0.20 in untreated and FTI-277-treated cells (n = 6 experiments), respectively.

Treatment with either the FTI or the GGTL had no apparent effect on the adhesion of the cells to the culture dish. No changes in cell number were seen after treating the cells with either the GGTL or the FTI. Agonist treatment did not significantly alter either the

Fig. 3. Summary of experiments demonstrating LPA-induced changes in the ratio of F- to G-actin in human airway smooth muscle cells pretreated with GGTL-2147. Data are expressed as means ± SE. Ratios of F- to G-actin (F:G actin) are shown for control cells and cells treated with the GGTL vehicle (DMSO), GGTL (GGTL-2147), LPA + DMSO, and LPA + GGTL-2147. LPA in the presence of DMSO induced a large and statistically significant increase in the F- to G-actin ratio that was completely blocked by the GGTL. The GGTL alone caused a decline in the F- to G-actin ratio, which most likely represented a decrease in the basal (unstimulated) activation of RhoA.

Fig. 4. Summary of experiments demonstrating endothelin-induced changes in the ratio of F- to G-actin in human airway smooth muscle cells pretreated with GGTL-2147. Data are expressed as means ± SE. Ratios of F- to G-actin are shown for control cells and cells treated with the vehicle (DMSO), the GGTL (GGTL-2147), endothelin alone, and endothelin + GGTL-2147. Endothelin induced a large and statistically significant increase in the F- to G-actin ratio that was abolished by pretreatment with GGTL-2147. In all experiments, treatment of cells with GGTL alone caused a decrease in the ratio of F- to G-actin, which presumably represented a decrease in the basal state of RhoA activation. Endo, endothelin.

Fig. 5. Summary of experiments showing carbachol-induced increases in the ratio of F- to G-actin. Data are expressed as means ± SE (n = 4 for all treatment groups). Treatment of the cells with GGTL-2147 reduced the baseline F- to G-actin ratio and completely blocked carbachol-induced increases in F- to G-actin. Data are shown for untreated cells (control), cells pretreated with GGTL-2147 (GGTL), cells treated with carbachol alone, and cells pretreated with GGTL-2147 and subsequently treated with carbachol (Carb-GGTL).

Fig. 6. Summary of experiments demonstrating LPA- and endothelin-induced changes in the ratio of F- to G-actin in human airway smooth muscle cells pretreated with FTI-277. Data are expressed as means ± SE. Ratios of F- to G-actin are shown for control cells and cells treated with the vehicle (DMSO), the FTI (FTI-277), LPA alone, LPA + FTI-277 pretreatment, endothelin alone, and endothelin + FTI-277. Both LPA and endothelin induced a statistically significant increase in the F- to G-actin ratio in the presence and absence of FTI-277 pretreatment. Treatment of the cells with FTI-277 alone caused no significant decrease in the ratio of F- to G-actin.
cell morphology or adhesion to the culture substrate. The reversibility of this treatment was not investigated.

To exclude the possibility that the GGTI blocked agonist-induced actin rearrangements by inhibiting heterotrimeric G protein function, carbachol-mediated inhibition of forskolin-stimulated adenyl cyclase activity was measured in the cells that were untreated or pretreated with GGTI-2147. Forskolin-stimulated adenyl cyclase activity was significantly inhibited by 1 μM carbachol in untreated cells and in cells treated for 72 h with either 10 μM GGTI-2147 or the vehicle (0.1% DMSO) (Fig. 7). In untreated cells, carbachol reduced adenyl cyclase activity to 79 ± 7.7% of forskolin-stimulated activity. In cells treated for 72 h with GGTI-2147, carbachol reduced adenyl cyclase activity to 69 ± 9.5%, and in cells treated with vehicle (0.1% DMSO), carbachol reduced forskolin-stimulated adenyl cyclase activity to 74 ± 15% (P > 0.05 between untreated, DMSO-treated, and GGTI-2147-treated cells). These results suggest that the function of the heterotrimeric G protein (G_i) was maintained despite 72 h of exposure to GGTI-2147.

**DISCUSSION**

The major finding of this study is that treatment of cultured human airway smooth muscle cells with either of two GGTIs (GGTI-286 or GGTI-2147) abrogated LPA- and endothelin-induced increases in actin reorganization, as reflected by the fluorescence ratio of F- to G-actin. In contrast, both agonists significantly increased the F- to G-actin fluorescence ratios in cells that were treated with an FTI. These data strongly suggest that the inhibition of endothelin- or LPA-induced increases in the F- to G-actin fluorescence ratio results from inhibition of geranylgeranyltransferase I rather than from a nonspecific effect of the drug or an effect on Ras-family proteins, which are posttranslationally farnesylated. Forskolin-stimulated adenyl cyclase activity was inhibited by carbachol in GGTI-treated cells; therefore, we conclude that the inhibition of LPA- and endothelin-induced increases of the F- to G-actin fluorescence ratio in GGTI-treated cells results from GGTI inhibition of RhoA function and not from an effect on the G_y subunit of G_i protein. Most G_y subunits are posttranslationally geranylgeranylated (rather than farnesylated), and inhibition of geranylgeranyltransferase I may potentially have an effect on coupling of the heterotrimeric G proteins to their downstream effectors, by interrupting G_y subunit function (30). We cannot, however, exclude the possibility that the heterotrimeric G_bγ subunit dimer mediating coupling between the M_2 muscarinic cholinergic receptor and G_i protein in our cells contains a farnesylated G_y subunit. Farnesyl-G_y subunits have been identified, but they are less potent stimulators of G_i protein than are geranylgeranyl-G_y subunits (30).

Potent and selective inhibitors of both farnesyltransferase and geranylgeranyltransferase I have been used in previous studies to determine the physiological role of farnesylation or geranylgeranylation, respectively, in cell signaling (6, 40). Two specific concerns arise when treating cells with these compounds: the possibility that nonspecific or alternative prenylation occurs when either the geranylgeranyltransferase I or the farnesyltransferase is inhibited, and the possibility that several prenylated proteins mediate specific functions in any signaling cascade, making it difficult to conclude that an observed effect results from inhibiting the prenylation of only one protein. Although RhoB, N-Ras, or K-Ras4B can variably be geranylgeranylated or farnesylated when its native prenyltransferase is inhibited (3, 8, 26, 39), alternative prenylation has not been demonstrated for RhoA. Plasmids containing RhoA constructs with recombinant COOH-terminal CAAX motifs (that direct either farnesylation or geranylgeranylation) have recently been expressed in NIH/3T3 cells, and the results suggest that even when RhoA is farnesylated, it is functionally indistinguishable from geranylgeranyl-conjugated RhoA (the native species) (1).

The present study in airway smooth muscle cells agrees with the study by Allal et al. (1) that concluded that RhoA prenylation (either farnesylation or geranylgeranylation) was necessary for stress fiber formation in NIH/3T3 cells. A recent study in mouse neuroblastoma cells, however, concluded that membrane translocation of RhoA was unnecessary for stress fiber formation (24). The authors of the latter study overexpressed a dominant negative isoform of RhoA in their cells rather than using a GGTI or a nonprenylated RhoA species, which may explain the conflicting results. Prenyltransferase inhibition was not measured directly in these experiments. However, the doses of the GGTI used in this study and the incubation times used were identical to those that have been published.
Previous studies from our laboratory have demonstrated that inhibition of RhoA activation with C3 exotoxin (which ADP-ribosylates asparagine-46 and renders RhoA inactive) blocked actin reorganization as measured by increases in F- to G-actin ratios in human airway smooth muscle cells stimulated with LPA, carbachol, or endothelin (17, 43). These studies as well as studies by other groups suggest that the activation of RhoA is necessary and sufficient to induce actin reorganization/stress fiber formation in many cell types (37). In addition to the inhibition of endothelin and LPA-induced alterations in actin reorganization, both cells treated with GG蒂-2147 and those treated with GG蒂-286 had a decreased ratio of F- to G-actin in the absence of agonist (see Figs. 3 and 4). This most likely represents a basal level of RhoA activation (and membrane translocation) that was inhibited by both GG蒂s; decreases in basal tension have been observed in vascular smooth muscle treated with a Rho kinase inhibitor (Y-27632), an observation that is similarly attributed to a basal activation of RhoA (13). Treatment of cells with the FTI (FTI-277) also decreased the agonist-induced changes in the F- to G-actin fluorescence ratio, although not as completely as with the GG蒂. This observation may result from functional interactions between Ras (a farnesylated protein) and RhoA. Ras potently activates RhoA through several indirect pathways including activation or inactivation of the p120GAP:p190RhoGAP complex (13, 47) (41) or by indirect activation of RhoA itself through Rac (a geranylgeranylated G protein) (22, 38).

In this study, fluorescence microscopy and dual-fluorophore labeling were used to quantify the relative changes in F- to G-actin, according to a method adapted from Knowles and McCullough (23). This technique has been validated in numerous studies over the past 10 years (10, 12, 29, 43). FITC-phalloidin selectively labels F-actin, and DNase I selectively labels G-actin, allowing for the simultaneous observation of both F- and G-actin in a single cell. Concurrent labeling of F- and G-actin, in addition to fluorescent labeling of the total protein content, has been used to normalize differences in cell size or the number of cells per image (16, 23, 42). Previous studies have shown a correlation between the total cellular actin concentration, as measured by quantitative fluorescence, and the DNase inhibition assay (35). Variations in the fluorescence intensity of both F- and G-actin occur between individual experiments. From the standpoint of data interpretation, however, these differences were minimized by the inclusion of both control and experimental groups on the same slide. There appeared to be some heterogeneity among cells in the treatment groups when examined individually; however, the data we present are averaged fluorescence intensities and indicate unambiguous trends in actin organization with the various treatment protocols. Cells on the same microscope slide invariably undergo identical conditions of culture, staining, and videomicroscopy. Furthermore, F- to G-actin fluorescence intensity data have been expressed as a ratio, abrogating the effects of different cell numbers on different slides.

Inhibition of the geranylgeranyl modification of the heterotrimeric G protein \( \gamma \)-subunit could potentially uncouple signaling from the endothelin or LPA receptor to RhoA and mimic an independent inhibitory effect of RhoA inactivation. Because heterotrimeric G protein activation precedes monomeric G protein activation, interference with heterotrimeric G protein function could potentially interrupt signaling between the G protein-coupled receptor and RhoA. A GG蒂 would theoretically inhibit the prenylation of geranylgeranyl-modified \( \gamma \) subunits, and alterations in \( \gamma \) subunit prenylation have been shown to alter effector functions including activity of adenyl cyclase (30). Membrane-associated G protein content is reduced by cholesterol loading of smooth muscle cells (due to decreased prenyl synthesis and geranylgeranylation of \( \gamma \) subunit), and these cells demonstrate a decreased response to G protein-mediated signaling events (34). We hypothesized that if geranylgeranyltransferase inhibition had a significant effect on the \( \gamma \) subunit, then carbachol signaling would be influenced because it is known to signal through \( \gamma_{\text{GTP}} \) protein in these cells (20). Data from the present study, however, convincingly demonstrate that carbachol-induced inhibition of forskolin-activated adenyl cyclase was unaffected by treatment with the GG蒂. Moreover, data published by our laboratory previously demonstrated that inactivation of \( \gamma \) subunit alone was insufficient to block the actin reorganization induced by either endothelin or LPA (17), but downregulation of \( \gamma \) protein blocked carbachol-induced signaling in these cells (18). Because the \( \gamma \) subunit is required for agonist-induced activation of the heterotrimeric G protein adenyl cyclase activity, we presume that the effects of the GG蒂 on heterotrimeric G protein \( \gamma \)-subunits would be reflected in the ability of carbachol to inhibit forskolin-activated adenyl cyclase activity. The apparent discrepancy between the effect of GG蒂-286 and GG蒂-2147 on \( \gamma \) subunit and RhoA isoprenylation may be explained by differences in half-lives of the two proteins. Prenylation (and the subsequent methylation that follows prenylation) increases the half-life of RhoA from 12 to 31 h (4). Similar data are not available for the subunits of heterotrimeric G proteins. At 72 h, a functionally significant proportion of RhoA may be unprenylated, whereas the data we have presented suggest that a physiologically insignificant proportion of the \( \gamma \) subunit is affected by geranylgeranyltransferase I inhibition at this time point.

This study demonstrates a role for RhoA isoprenylation in endothelin- or LPA-induced actin reorganization. We show that inhibition of geranylgeranyltransferase I with specific inhibitors blocks RhoA activation in response to endothelin or LPA but appears to leave heterotrimeric G protein function unaltered. These data demonstrate, for the first time in human airway smooth muscle cells, that selective GG蒂s can inhibit...
agonist-induced actin reorganization, and, presumably, other signaling targets of RhoA.

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REFERENCES


