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3-Hydroxy-3-methylglutaryl CoA reductase inhibitors up-regulate transforming growth factor-β signaling in cultured heart cells via inhibition of geranylgeranylation of RhoA GTPase

HO-JIN PARK AND JONAS B. GALPER*

Cardiovascular Research Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115

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ABSTRACT **Transforming growth factor-β (TGFβ) signaling has been shown to play a role in cardiac development as well as in the pathogenesis of cardiovascular disease. Prior studies have suggested a relationship between cholesterol metabolism and TGFβ signaling. Here we demonstrate that induction of the cholesterol metabolic pathway by growth of embryonic chicken atrial cells in medium supplemented with lipoprotein-depleted serum coordinately decreased the expression of the TGFβ type II receptor (TGFβRII), TGFβ$_1$, and TGFβ signaling as measured by plasminogen activator inhibitor-1 (PAI-1) promoter activity. Inhibition of the cholesterol metabolic pathway by the hydrophobic 3-hydroxy-3-methylglutaryl CoA (HMGCoA) reductase inhibitors, simvastatin and atorvastatin, reversed the effect of lipoprotein-depleted serum and up-regulated TGFβRII expression, whereas the hydrophilic HMGCoA reductase inhibitor, pravastatin, had no effect. Simvastatin stimulated the expression of TGFβRII, TGFβ$_1$, and PAI-1 at the level of transcription. Experiments using specific inhibitors of different branches of the cholesterol metabolic pathway demonstrated that simvastatin exerted its effect on TGFβ signaling by inhibition of the geranylgeranylation pathway. C3 exotoxin, which specifically inactivates geranylgeranylated Rho GTPases, mimicked the effect of simvastatin on PAI-1 promoter activity. Cotransfection of cells with a PAI-1 promoter-reporter and a dominant-negative RhoA mutant increased PAI-1 promoter activity, whereas cotransfection with a dominant-active RhoA mutant decreased PAI-1 promoter activity. These data support the conclusion that TGFβ signaling is regulated by RhoA GTPase and demonstrate a relationship between cholesterol metabolism and TGFβ signaling. Our data suggest that in patients treated with HMGCoA reductase inhibitors, these agents may exert effects independent of cholesterol lowering on TGFβ signaling in the heart.**

Transforming growth factor-β (TGFβ) signaling plays an important role in cardiac development, cardiac hypertrophy, ventricular remodeling, and the early response to myocardial infarction (1–2). Data that correlate severe coronary artery disease with levels of circulating activated TGFβ suggest that TGFβ signaling might also play a role in atherogenesis (3–5). Studies that demonstrate that TGFβ is capable of regulating the expression of low-density lipoprotein (LDL) receptors and the incorporation of ^{14}C-acetate into cholesterol suggest that TGFβ signaling might play a role in regulating cholesterol metabolism (6, 7). Furthermore, in aortas of cholesterol-fed Watanabe rabbits, levels of TGFβ$_1$ are increased (8). These data suggest a relationship between cholesterol metabolism, TGFβ signaling, and cardiovascular disease.

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The cholesterol metabolic pathway may be stimulated by depriving cells of lipoproteins and inhibited by 3-hydroxy-3-methylglutaryl CoA (HMGCoA) reductase inhibitors (9). Farnesylpyrophosphate (FPP) represents a branchpoint in the cholesterol metabolic pathway. Not only does it serve as a precursor to cholesterol, but it is also a precursor to ubiquinone, dolichol phosphate, and geranylgeranylpyrophosphate (GGPP), which is required for the posttranslational lipidation and membrane localization of small GTP-binding proteins such as Rho family members. FPP itself is required for the lipidation and membrane localization of Ras (10, 11).

Small GTP-binding proteins play a major role in the regulation of the cell cycle and in the control of gene expression (10, 11). We have recently shown that the posttranslational lipidation of membrane-associated proteins is a regulatable process (12). Thus induction of the cholesterol metabolic pathway increased the extent of lipidation and membrane localization of Ras and the expression of proteins involved in second messenger pathways. Inhibition of the cholesterol metabolic pathway by HMGCoA reductase inhibitors interfered with farnesylation and membrane localization of Ras and reversed the effects of induction of the cholesterol metabolic pathway on gene expression (12–14).

TGFβ signaling is mediated via the interaction of two different TGFβ receptor subtypes, both containing serine/threonine kinase domains (15, 16). On binding of TGFβ to the TGFβ type II receptor (TGFβRII), the receptor is autophosphorylated. In the presence of ligand, TGFβRII forms a complex with the TGFβ type I receptor and catalyzes its phosphorylation. The TGFβRI then interacts with downstream signaling factors such as Smad proteins. The plasminogen activator inhibitor-1 (PAI-1) promoter is one of the major targets of Smad proteins.

The use of HMGCoA reductase inhibitors to study the regulation of cholesterol metabolism and TGFβ signaling has important clinical implications (17). HMGCoA reductase inhibitors are in wide clinical use for the treatment and prevention of coronary artery disease. Recent data have suggested that these drugs might have effects on coronary risk and cellular physiology that are independent of cholesterol lowering (17, 18). Six HMGCoA reductase inhibitors are currently in clinical use (19, 20). They demonstrate markedly different hydrophobicities: simvastatin is the most hydrophobic and pravastatin the most hydrophilic. Several studies have suggested that these differences in hydrophobicity might be related to differences in the ability of the HMGCoA reductase

Abbreviations: LDL, low-density lipoprotein; HMGCoA, 3-hydroxy-3-methylglutaryl CoA; TGFβ, transforming growth factor-β; TGFβRII, TGFβ type II receptor; LPDS, lipoprotein-depleted serum; PAI-1, plasminogen activator inhibitor-1; FPP, farnesylpyrophosphate; GGPP, geranylgeranylpyrophosphate.

*To whom reprint requests should be addressed at: Cardiovascular Research Division, Department of Medicine, Thorn Building No. 1209, Brigham and Women's Hospital and Harvard Medical School, 75 Francis Street, Boston, MA 02115. E-mail: galper@calvin.bwh.harvard.edu.

inhibitors to mediate nonlipid-lowering effects (19). Several studies have suggested that HMGCoA reductase inhibitors alter the expression of TGFβ_1 (21, 22). We report here a relationship between cholesterol metabolism and TGFβ signaling in cultured embryonic chicken atrial cells. In these cells, inhibition of the cholesterol metabolic pathway by HMGCoA reductase inhibitors induces the coordinate up-regulation of the expression of TGFβRII and its ligand, TGFβ_1, and an increase in TGFβ signaling. This effect is independent of cholesterol lowering and is caused by interference of the hydrophobic HMGCoA reductase inhibitors with the post-translational geranylgeranylation of a member of the Rho family of small GTP-binding proteins.

MATERIALS AND METHODS

Reagents. Cell culture media and supplies were from Life Technologies (Grand Island, NY). A monoclonal antibody to TGFβRII was from Transduction Laboratories (Lexington, KY) and an anti-human c-myc antibody was from PharMingen. Pravastatin, atorvastatin, and simvastatin were gifts from Bristol-Myers Squibb. The squalene synthase inhibitor, TMD, was a kind gift from Thomas Spencer (Dartmouth College, Hanover, NH) (23); FTI-277, an inhibitor of farnesyltransferase (24), and GGTI-298, an inhibitor of geranylgeranyltransferase I (25), were kind gifts from Said Sebti (University of South Florida, Tampa, FL).

Plasmids. pTGFβRII-500/36-Lux and phTG5 were gifts from Seong-Jin Kim (Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD) (26, 27) and p3TP-Lux containing the putative TGFβ responsive region of the human PAI-1 promoter was a gift from Joan Massague (Memorial Sloan–Kettering Cancer Center, NY) (28). phTGFβ5-Lux was generated by subcloning a smaller *Kpn*I-*Bam*HI fragment of phTG5 into *Kpn*I-*Bam*HI sites of pXP2, a promoterless luciferase vector (12). pGEX2F-C3 was a gift from Larry Feig (Tufts University, Boston, MA) (29). pRK5 myc-L63RhoA, pCDNA3 myc-N19RhoA, and pEFmyc-C3 were gifts from Alan Hall (University College London). pCMVβgal was from CLONTECH.

Preparation of Lipoprotein-Depleted Serum (LPDS). LPDS was prepared as described previously (12).

Primary Culture of Chicken Embryonic Heart Cells. Heart cells were cultured from embryos 14 days *in ovo*. Atrial cultures were prepared by a modification of the method of DeHaan (30) as described previously (14).

Purification of C3 Exotoxin. C3 exotoxin was overproduced in *Escherichia coli* transformed with pGEX2F-C3 and purified as described by Dillon and Feig (29).

Immunoblot Analysis. Equal amounts of protein from extracts of cultured heart cells were subjected to SDS/PAGE, followed by electroblotting onto a polyvinylidene difluoride membrane (Schleicher & Schuell). Blots were probed with the indicated primary antibody followed by a secondary antibody conjugated with horseradish peroxidase and visualized by chemiluminescence.

Transfection of Cells. LipofectAMINE Plus (Life Technologies, Grand Island, NY) was used according to the manufacturer's protocol with slight modifications. A total of 2 μg of plasmid DNA, including 0.1 μg of pCMVβgal, was used for transfection of cells cultured in 60-mm dishes to 80% confluence. Cells were incubated with the liposome-DNA complex in medium supplemented with 6% Nu-serum for 5 h. Cells were washed and allowed to recover for 12 h in medium supplemented with 6% FCS. Medium was removed and cells incubated for 16 h, as indicated in the figure legends. Luciferase and β-galactosidase assays were carried out as described by Ausubel *et al.* (31). Data were presented as the mean value $\pm$SEM and analyzed by Student's *t* test where indicated.

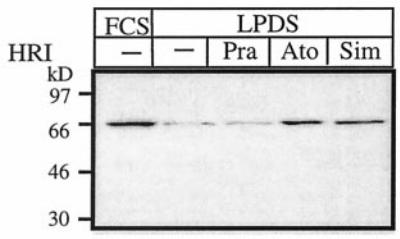

FIG. 1. Regulation of TGFβRII expression by the cholesterol metabolic pathway. Embryonic chicken atrial cells were cultured in media with either FCS or LPDS and treated with various HMGCoA reductase inhibitors each at 10 μM for 16 h. Lane 1, FCS; lane 2, LPDS; lane 3, LPDS plus pravastatin (Pra); lane 4, LPDS plus atorvastatin (Ato); lane 5, LPDS plus simvastatin (Sim). Thirty micrograms of crude cell extract were subjected to SDS/PAGE followed by immunoblotting with a TGFβRII antibody. Data are typical of four similar experiments.

RESULTS

Effect of Regulating the Cholesterol Metabolic Pathway on the Expression of TGFβRII in Cultured Heart Cells. To determine the effect of induction of the cholesterol metabolic pathway on TGFβ signaling, we measured levels of TGFβRII by immunoblot analysis of proteins from embryonic chicken atrial cells grown in media supplemented with either FCS or LPDS (Fig. 1). The level of TGFβRII was markedly decreased in cells grown in LPDS compared with that in cells grown in FCS. To determine whether inhibition of the cholesterol metabolic pathway by HMGCoA reductase inhibitors reversed the effect of LPDS on TGFβRII expression, cells were incubated in LPDS plus 10 μM either pravastatin, atorvastatin, or simvastatin. Both atorvastatin and simvastatin completely reversed the effect of LPDS on the expression of TGFβRII. Pravastatin had no effect on LPDS inhibition of TGFβRII expression (Fig. 1).

Effect of Regulating the Cholesterol Metabolic Pathway on TGFβRII and TGFβ_1 Promoter Activity. To determine whether the regulation of TGFβRII expression occurred at the level of transcription, TGFβRII promoter activity was measured in cells transiently transfected with a TGFβRII promoter–luciferase reporter. Growth in LPDS decreased TGFβRII promoter activity by 73 $\pm$ 7% (n = 4) compared with cells grown in FCS; simvastatin reversed this effect (Fig. 2).

TGFβ_1 promoter activity was measured in cells transfected with a TGFβ_1 promoter–luciferase reporter. Growth in medium supplemented with LPDS decreased TGFβ_1 promoter

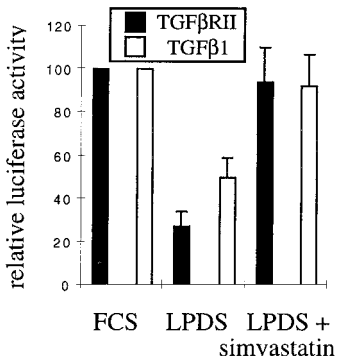

FIG. 2. Coordinate regulation of TGFβRII and TGFβ_1 promoter activity by control of the cholesterol metabolic pathway. Cells were transfected with either pTGFβRII-500/36-Lux (■) or phTGFβ5-Lux (□) plus pCMVβgal. After recovery, cells were incubated for 16 h in media with either FCS, LPDS, or LPDS plus simvastatin (20 μM). Luciferase activity was normalized to β-galactosidase activity. Data are plotted as the mean $\pm$ SEM of three independent experiments.

activity by 50 ± 9% ($n = 3$) compared with control. This effect was completely reversed by simvastatin (Fig. 2). Hence factors that regulate the cholesterol metabolic pathway coordinately regulate the expression of both TGFβRII and TGFβ_1.

Up-Regulation of TGFβRII and TGFβ_1 Expression and TGFβ Signaling by Simvastatin Is Mediated by Inhibition of the Geranylgeranylation Pathway. To identify which of the FPP-dependent pathways played a role in the regulation of TGFβRII and TGFβ_1 expression, TMD, an inhibitor of squalene synthase, was used to block cholesterol biosynthesis (23); FTI-277, an inhibitor of farnesyltransferase, to block protein farnesylation (24); and GGTI-298, an inhibitor of geranylgeranyltransferase, to block protein geranylgeranylation (25). Data summarized in Fig. 3*A* demonstrated that neither TMD nor FTI-277 had an effect on TGFβRII and TGFβ_1 promoter activities (Fig. 3*A*, lanes 3 and 4), whereas GGTI-298 increased TGFβRII and TGFβ_1 promoter activities by 4.5 ± 0.48-fold and 1.9 ± 0.16-fold ($n = 3$), respectively (Fig. 3*A*, lane 5). Similar results were obtained from immunoblot analysis of the expression of TGFβRII protein (Fig. 3*B*). Thus, the coordinate up-regulation of the expression of TGFβRII and TGFβ_1 by simvastatin is mediated by the inhibition of protein geranylgeranylation.

If simvastatin acts by inhibition of the geranylgeranylation pathway, addition of GGPP, the substrate for geranylgeranyltransferase, should reverse the up-regulation of TGFβRII expression induced by simvastatin. Incubation of cells in LPDS with simvastatin plus GGPP reversed the effect of simvastatin on TGFβRII promoter activity (Fig. 4*A*) and on TGFβRII

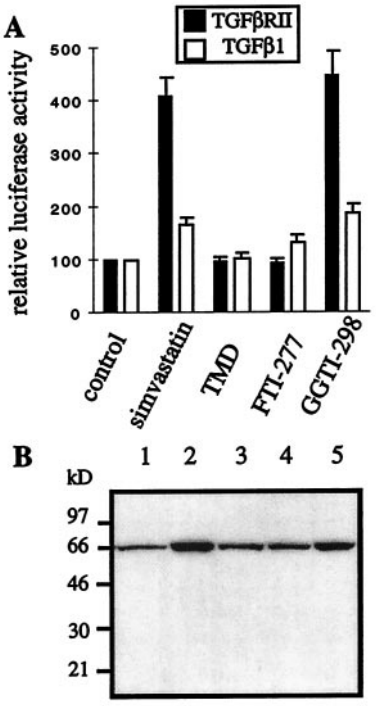

FIG. 3. Up-regulation of TGFβRII and TGFβ_1 expression by inhibition of the geranylgeranylation pathway. (*A*) Embryonic chicken atrial cells were transfected with either pTGFβRII-500/36-Lux or phTGFβ5-Lux plus pCMVβgal. Cells were allowed to recover followed by a 16-h incubation in media supplemented with LPDS and the various inhibitors. Lane 1, control; lane 2, 20 μM simvastatin; lane 3, 50 μM TMD; lane 4, 10 μM FTI-277; lane 5, 10 μM GGTI-298. Luciferase activity was normalized to β-galactosidase activity. Data are plotted as the mean ± SEM of three independent experiments. (*B*) Cells were grown in media with LPDS and treated with various inhibitors as described above. Thirty micrograms of crude cell extract were analyzed for the expression of TGFβRII protein using a TGFβRII antibody. Data are typical of three similar experiments.

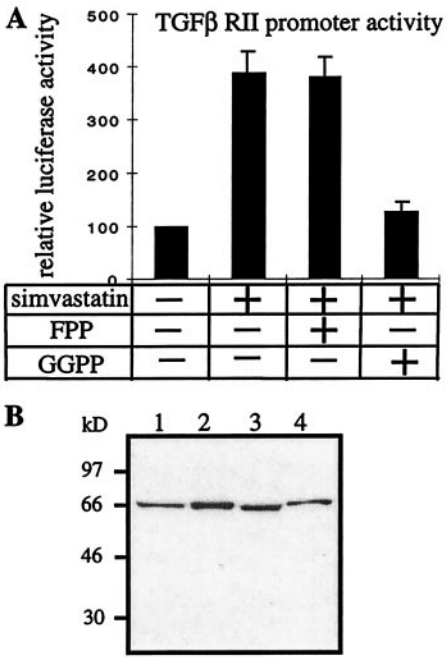

FIG. 4. GGPP reverses the effect of simvastatin on TGFβRII expression. (*A*) Embryonic chicken atrial cells were transfected with pTGFβRII-500/36-Lux plus pCMVβgal. After recovery, cells were incubated for 16 h in media with LPDS plus: lane 1, no additions; lane 2, 10 μM simvastatin; lane 3, 10 μM simvastatin plus 10 μM FPP; lane 4, 10 μM simvastatin plus 10 μM GGPP. Luciferase activity was normalized to β-galactosidase activity. Data are plotted as the mean ±SEM of four independent experiments. (*B*) Cells were grown in media supplemented with LPDS and treated as described above. Thirty micrograms of crude cell extract were analyzed by immunoblotting for the expression of TGFβRII protein. Data are typical of three similar experiments.

protein (Fig. 4*B*), whereas FPP had no effect.

To determine the physiological significance of the coordinate up-regulation of TGFβRII and TGFβ_1 expression, we studied the effect of regulating the cholesterol metabolic pathway on PAI-1 promoter activity (32). Growth of cells in LPDS decreased PAI-1 promoter activity by 71 ± 2% ($n = 4$) compared with cells cultured with FCS, whereas simvastatin reversed the effect of LPDS on PAI-1 promoter activity (Fig. 5*A*). This effect of simvastatin was dose dependent; 0.2 μM simvastatin increased PAI-1 promoter activity by 31 ± 2% ($n = 4$) compared with cells grown in LPDS alone ($P < 0.01$), whereas 1 μM simvastatin increased PAI-1 promoter activity by 210 ± 8% ($n = 4$).

To determine whether the effect of simvastatin on PAI-1 promoter activity was also mediated via an effect on the geranylgeranylation pathway, we compared the effect of TMD, FTI-277, and GGTI-298 on PAI-1 promoter activity. GGTI-298 mimicked the effect of simvastatin and increased PAI-1 promoter activity up to 5.5 ± 0.48-fold ($n = 4$), whereas TMD and FTI-277 showed no effect (Fig. 5*B*). GGPP, but not FPP, completely reversed the effect of simvastatin on PAI-1 promoter activity (Fig. 5*B*). Hence TGFβ signaling is regulated by the cholesterol metabolic pathway in parallel with the regulation of TGFβRII and TGFβ_1 via an effect on protein geranylgeranylation.

If induction of the cholesterol metabolic pathway by growth of cells with LPDS interferes with TGFβ signaling by increasing the availability of GGPP, then addition of GGPP to cells grown in the presence of an exogenous source of cholesterol might also inhibit PAI-1 promoter activity. Incubation of cells in medium supplemented with FCS plus 10 μM GGPP resulted in a 56 ± 2% decrease ($n = 3$) in PAI-1 promoter activity

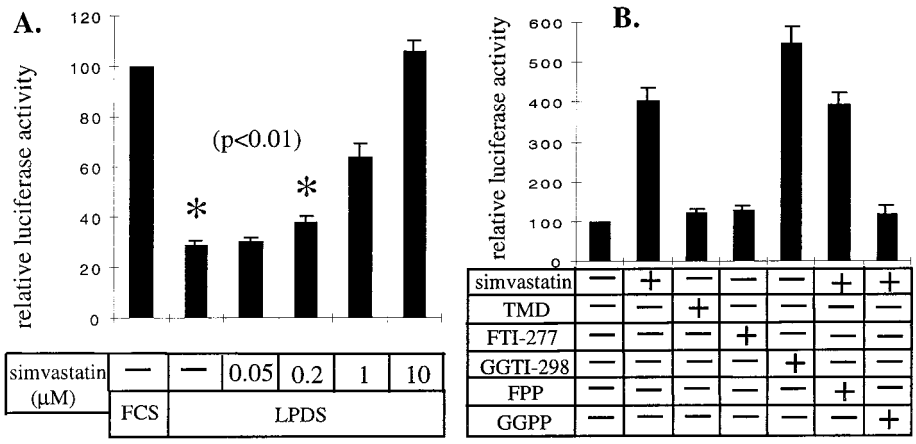

FIG. 5. Regulation of TGFβ signaling by control of the cholesterol metabolic pathway. Embryonic chicken atrial cells were transfected with p3TP-Lux plus pCMVβgal. After recovery, cells were incubated for 16 h in media supplemented with either (*A*) FCS, LPDS, or LPDS plus various concentrations of simvastatin (0–10 μM); (*B*) LPDS and either: no additions; 10 μM simvastatin; 50 μM TMD; 10 μM FTI-277; 10 μM GGTI-298; 10 μM simvastatin plus 10 μM FPP; 10 μM simvastatin plus 10 μM GGPP. Luciferase activity was normalized to β-galactosidase activity. Values are the mean ± SEM of four independent experiments.

compared with cells cultured in FCS alone (Fig. 6*A*). This decrease was similar to the effect of LPDS on PAI-1 promoter activity.

RhoA GTPase Regulates TGFβ Signaling. *Clostridium botulinum* C3 toxin specifically catalyzes the ADP ribosylation of members of the Rho family of small GTP-binding proteins and inhibits their function (33). Addition of C3 toxin to cultured embryonic chicken atrial cells resulted in a 2.7 ± 0.28-fold increase ($n = 3$) in TGFβRII promoter activity and a 4.5 ± 0.38-fold increase ($n = 3$) in PAI-1 promoter activity (Fig. 6*B*). These results implicate a member of the Rho family of small GTP-binding proteins in the regulation of TGFβRII and PAI-1 promoter activity.

To further establish which member of the Rho family played a role in the regulation of TGFβ signaling, cells were cotransfected with a PAI-1 promoter reporter and a vector expressing either a dominant-active RhoA mutant, a dominant-negative RhoA mutant, or a vector containing the C3 exotoxin gene. Each of these constructs was tagged at the N terminus with a myc-epitope. Cells transfected with the dominant-active mutant of RhoA demonstrated a 37 ± 9% decrease ($n = 3$) in PAI-1 promoter activity compared with control ($P < 0.01$). Cotransfection with a dominant-negative mutant resulted in a 5.8 ± 0.62-fold increase ($n = 3$) in PAI-1 promoter activity whereas cotransfection with C3 toxin resulted in a 4.8 ± 0.76-fold ($n = 3$) increase (Fig. 7*A*). Data in Fig. 7*B* demonstrate that each of these genes was expressed in cultured chicken atrial cells as detected by immunoblot analysis by using an anti-myc antibody. These results indicate that RhoA GTPase may regulate TGFβ signaling.

DISCUSSION

Several studies have suggested a possible relationship between $TGF\beta_1$ expression and cholesterol metabolism. An increase in expression of $TGF\beta_1$ has been observed in balloon-injured carotid arteries of rabbits treated with the HMGCoA reductase inhibitor NK-104, while the HMGCoA reductase inhibitor lovastatin decreased $TGF\beta_1$ expression in glomeruli of diabetic rats (21, 22). Furthermore, cholesterol feeding has been shown to effect $TGF\beta_1$ expression in aortas of Watanabe rabbits (8). These results appear to be cell-type specific and do not demonstrate an effect on TGFβ signaling. Data presented here extend these studies by demonstrating a relationship between cholesterol metabolism and TGFβ signaling. The induction of the cholesterol metabolic pathway by growth of embryonic chicken atrial cells in the absence of lipoproteins resulted in a coordinate decrease in the expression of TGFβRII, $TGF\beta_1$, and TGFβ signaling as measured by a decrease in PAI-1 promoter activity. Conversely, inhibition of the cholesterol metabolic pathway by the hydrophobic HMGCoA reductase

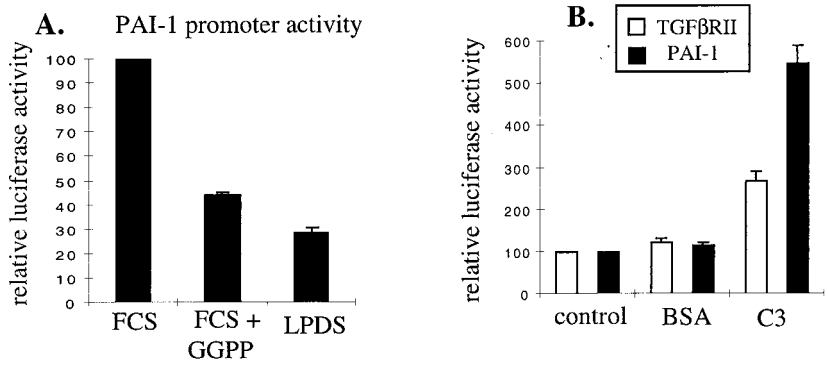

FIG. 6. Regulation of TGFβ signaling via the geranylgeranylation pathway. (*A*) Cells were transfected with p3TP-Lux plus pCMVβgal. After recovery, cells were incubated for 16 h in media with: lane 1, FCS alone; lane 2, FCS plus 10 μM GGPP; lane 3, LPDS alone. Luciferase activity was normalized to β-galactosidase activity. Data are plotted as the mean ± SEM of three independent experiments. (*B*) Cells were transfected with either pTGFβRII-500/36-Lux or p3TP-Lux plus pCMVβgal. After recovery, cells were cultured for 16 h in media supplemented with LPDS plus: lane 1, control; lane 2, 50 μg BSA/ml; lane 3, 50 μg C3 exotoxin/ml. Luciferase activity was normalized to β-galactosidase activity. Values are the mean ± SEM of three independent experiments.

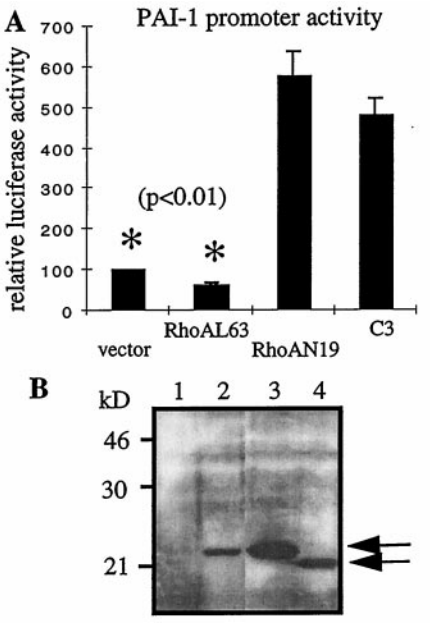

FIG. 7. Regulation of TGFβ signaling by RhoA GTPase. (*A*) Embryonic chicken atrial cells were cotransfected with p3TP-Lux, pCMVβgal, and either pCDNA3, pRK5 myc-RhoA L63, pCDNA3 myc-RhoA N19, or pEFmyc-C3. After recovery, cells were cultured for 16 h in media with LPDS. Luciferase activity was normalized to β-galactosidase activity. Values are the mean ± SEM of three independent experiments. (*B*) Thirty micrograms of crude cell extract from cells were analyzed for the expression of RhoA mutants and C3 toxin by immunoblotting using a myc antibody. Data are typical of three similar experiments.

inhibitors markedly increased the expression of TGFβRII, $TGF\beta_1$, and TGFβ signaling.

Geranylgeranylation is required for the membrane localization and functioning of small GTP-binding proteins such as Rho family members. Previous data have demonstrated that posttranslational lipidation might be a regulatable process. Thus induction of the cholesterol metabolic pathway was capable of increasing the farnesylation and membrane localization of Ras (12). The finding that GGPP, the substrate for geranylgeranyltransferase, not only reversed the effect of simvastatin on TGFβ signaling, but also mimicked the effect of induction of the cholesterol metabolic pathway on TGFβ signaling in cells cultured in FCS, supports the conclusion that increased substrate availability for geranylgeranyltransferase caused by either induction of the cholesterol metabolic pathway or exogenously added GGPP inhibits the expression of TGFβRII and $TGF\beta_1$.

The finding that overexpression of a dominant-active RhoA mutant mimicked the effect of LPDS (induction of the cholesterol metabolic pathway) on TGFβ signaling and a dominant-negative RhoA mutant mimicked the effect of HMGCoA reductase inhibitors on TGFβ signaling supports the conclusion that RhoA GTPase is the geranylgeranylated protein that negatively regulates TGFβ signaling. Atfi *et al.* and Musci *et al.* demonstrated that the response of the PAI-1 promoter to exogenously added TGFβ was inhibited by dominant-negative mutants of Rho family members, suggesting that Rho positively regulated TGFβ signaling (34, 35). Our studies, carried out in the absence of exogenously added TGFβ, measured the effects of regulating the cholesterol metabolic pathway on the function of an autocrine loop for TGFβ signaling. In contrast to those studies, our data support the existence of a negative control of TGFβ signaling by RhoA. These differences could be cell-type specific. Taken together, these data suggest a new mechanism for the control of $TGF\beta_1$ and TGFβRII expression and TGFβ signaling via the regulation of RhoA GTPase function.

Prior studies have suggested that TGFβ regulates the cholesterol metabolic pathway via an effect on the expression of LDL receptors (6, 7). In bovine adrenocortical cells, TGFβ interfered with steroidogenesis in parallel with a decrease in the level of LDL receptors (36). TGFβ has also been shown to stimulate the expression of LDL receptors in HepG2 cells and human mesangial cells and decrease the incorporation of [^{14}C]acetate into cholesterol (7, 37). Because GGPP and FPP are products of the cholesterol metabolic pathway, a decrease in endogenous cholesterol production because of TGFβ induction of LDL receptor number should decrease GGPP and FPP levels. Hence, TGFβ might be expected to interfere with the geranylgeranylation and farnesylation of small GTP-binding proteins by an effect on substrate availability. In support of this conclusion, recent studies from our laboratory demonstrate that in cultured embryonic chicken atrial cells, TGFβ regulates the lipidation of Ras by FPP (S. M. Ward and J.B.G., unpublished results). Hence, if as suggested by the data presented here a Rho family member regulates TGFβ signaling, and if TGFβ signaling regulates availability of substrates for the lipidation of small GTP-binding proteins, a feedback loop might exist by which TGFβ might regulate its own expression via control of protein geranylgeranylation.

Recent studies have demonstrated that HMGCoA reductase inhibitors decrease coronary events in patients suffering from coronary artery disease (17, 18). Further analysis has suggested that cholesterol reduction alone does not appear to fully account for the decrease in coronary events (38). The finding of a relationship between the regulation of cholesterol metabolism and TGFβ signaling suggests that alterations in TGFβ signaling in response to HMGCoA reductase inhibitors may be responsible for some of the therapeutic effects of these agents.

The finding that HMGCoA reductase inhibitors, simvastatin and atorvastatin, but not pravastatin, are capable of inducing an increase in the expression of TGFβRII could have important implications for the mechanism of action of these agents. Although HMGCoA reductase inhibitors are structurally quite similar, they differ markedly in hydrophobicity: simvastatin > atorvastatin > pravastatin (19, 20, 39). Although all three of these agents are transported into the liver, uptake into nonliver cells depends on relative hydrophobicity. Hence it is likely that differences in hydrophobicity are responsible for the finding that only simvastatin and atorvastatin effected the expression of TGFβRII in cultured heart cells. Therapeutic doses of simvastatin and pravastatin have been shown to result in serum levels of 0.02–0.27 μM and 0.09–0.16 μM, respectively (40). These concentrations are similar to those found to have significant effects on TGFβ signaling in cultured chicken atrial cells reported here.

Based on the data presented here, the effects of cholesterol-lowering therapy on TGFβ signaling might be expected to differ depending on whether patients are treated by the dietary restriction of cholesterol, a hydrophilic HMGCoA reductase inhibitor such as pravastatin, or hydrophobic HMGCoA reductase inhibitors such as simvastatin or atorvastatin. Although the role of TGFβ in atherogenesis remains controversial, differences in effects on TGFβ signaling could be important clinical distinctions between different classes of HMGCoA reductase inhibitors and different modes of cholesterol-lowering therapy.

PAI-1 plays a role in the inhibition of thrombolysis and smooth muscle cell migration and in increasing the stability of atherosclerotic plaques (41). Thus a change in PAI-1 expression in response to HMGCoA reductase inhibitors could have important clinical implications. A number of studies have attempted to determine the effect of HMGCoA reductase inhibitors on levels of PAI-1 in hypercholesterolemic patients. Patients treated with simvastatin and atorvastatin demon-

strated an increase in PAI-1 activity, whereas pravastatin treatment was associated with a decrease in PAI-1 (20, 42, 43). A recent study using SV40-transformed rat aortic endothelial cells demonstrated a decrease in PAI-1 activity in response to lovastatin (44). These effects of HMGCoA reductase inhibitors are likely to depend on cell type and cell density and may be different in transformed cells.

Both TGFβ and HMGCoA reductase inhibitors have been shown to interfere with cell division and cellular migration (25, 45, 46). HMGCoA reductase inhibitors have been shown to induce the expression of $p21^{WAF1/C1P1}$, which negatively regulates cell-cycle progression by inhibiting cyclin-dependent kinase activity (25, 46). Both HMGCoA reductase inhibitors and GGTI-298 were shown to regulate $p21^{WAF1/C1P1}$ expression via an effect on a TGFβ response element in the upstream region of the $p21^{WAF1/C1P1}$ promoter (46, 47). Taken together with our data that demonstrate that both simvastatin and GGTI-298 increase TGFβ signaling, these data support the conclusion that HMG-CoA reductase inhibitors regulate $p21^{WAF1/C1P1}$ expression and the cell cycle via the stimulation of TGFβ signaling.

The role of an increase in TGFβ signaling in cardiomyocytes in response to HMGCoA reductase inhibitors is unclear. TGFβ signaling has been associated with cardiac hypertrophy, the early response to myocardial infarction, ventricular remodeling, and hypertrophic cardiomyopathy (1–2). Hypertrophic cardiomyopathy has been associated with an increase in TGFβ_1 expression and in the number of TGFβ receptors (48). In a pressure-loaded model for cardiac hypertrophy in which rats were subjected to abdominal aortic constriction or subcutaneous norepinephrine, cardiac myocytes derived from the hearts of these animals demonstrated an increase in TGFβ_1 (49). In acute myocardial infarction 24–48 hr after ligation of the left coronary, artery levels of TGFβ_1 mRNA increased 2- to 4-fold compared with controls (2). Our data suggest the intriguing hypothesis that treatment with the more hydrophobic HMGCoA reductase inhibitors might exert an effect on the pathogenesis of these processes and contribute to their progression by stimulating the TGFβ signaling pathway.

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