Expression of Connective Tissue Growth Factor in Human Renal Fibroblasts: Regulatory Roles of RhoA and cAMP

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Abstract. The induction of connective tissue growth factor (CTGF) was investigated in a human renal fibroblast cell line that exhibited many characteristics of primary human renal fibroblasts. Induction of CTGF mRNA was observed after treatment of the cells with transforming growth factor-β (TGF-β) or, even more prominently, lysophosphatidic acid (LPA). LPA induced a rapid transient increase in CTGF mRNA expression, with maximal levels being observed after 1 to 2 h. This increase was accompanied by CTGF protein synthesis. Induction of CTGF was insensitive to pertussis toxin and was not dependent on the activation of p42/44 mitogen-activated protein kinases. Inhibition of the proteins of the Rho family with toxin B from Clostridium difficile abrogated basal and LPA-mediated induction of CTGF. Specific targeting of RhoA with C3 exotoxin or of the Rho kinases with the inhibitor Y-27632 similarly prevented induction of CTGF, implicating RhoA as a signaling module downstream of LPA. Inhibition of RhoA depolymerized the actin cytoskeleton, as did treatment with cytochalasin D. Preincubation of the human renal fibroblasts with cytochalasin D prevented induction of CTGF by LPA, indicating a strong contribution of an intact cytoskeleton. Interference with RhoA signaling similarly inhibited the induction of CTGF by TGF-β. Elevation of intracellular levels of cAMP and thus activation of protein kinase A prevented induction of CTGF expression. The cytoskeletal effects of cAMP, however, were reversed by LPA. These data indicate complex interactions involving LPA-mediated activation of RhoA- and protein kinase A-dependent signaling pathways. The data thus demonstrate the regulatory functions of the small GTPase RhoA and of an intact cytoskeleton in the expression of CTGF after stimulation with LPA or TGF-β. Analogous signal transduction pathways were previously demonstrated in rat mesangial cells, suggesting a more general role for RhoA in the regulation of CTGF expression.

Connective tissue growth factor (CTGF) was first purified from conditioned medium of human umbilical vein endothelial cells and was demonstrated to account for much of the bioactivity previously attributed to platelet-derived growth factor (1). Attention has since been drawn to CTGF as a downstream mediator of transforming growth factor-β (TGF-β) (2). CTGF expression is strongly increased by TGF-β in fibroblasts (3). Regulation occurs at the transcriptional level; a novel, specific, TGF-β response element was identified in the CTGF promoter (4). CTGF mediates TGF-β-induced anchorage-independent growth in a continuous line of cultured normal rat kidney fibroblasts (5,6). It also mediates TGF-β-induced synthesis of type I collagen and fibronectin (7). In line with these in vitro findings, elevated levels of TGF-β and CTGF are observed during wound repair (3), indicating a functional role for CTGF in repair processes.

Elevated levels of CTGF are also observed in fibrotic lesions (8–10), and CTGF is suggested to be functionally involved in the development and progression of fibrotic diseases. In the kidney, CTGF mRNA levels were elevated in the majority of biopsies obtained from patients with various types of renal diseases characterized by glomerulosclerosis and tubulointerstitial fibrosis (11). Expression of CTGF in diabetic glomerulosclerosis was recently confirmed in the db/db mouse model (12). Furthermore, enhanced CTGF expression was detected in human and rat mesangial cells when the cells were stimulated with TGF-β or exposed to elevated levels of glucose (12,13). In addition to mesangial cells, CTGF exhibited positive staining in renal epithelial cells and interstitial fibroblasts in human biopsies (11). Most of the interstitial fibroblasts were characterized as myofibroblasts by the expression of α-smooth muscle actin. These fibroblasts are considered to play a key role in renal interstitial fibrosis by secreting extracellular matrix components, which lead to disorganization of the normal organ architecture and loss of function (reviewed in References 14–16).

To date, TGF-β has been characterized as the main growth factor inducing CTGF, with little effect of other growth factors such as platelet-derived growth factor or fibroblast growth factor (4). Very recent studies indicated that, depending on the cell type, bioactive peptides such as thrombin, factor VIIa, or des-Arg9-kallidin or low-molecular weight mediators such as serotonin or lysophosphatidic acid (LPA) might also induce
CTGF expression (16–18). Signaling pathways leading to CTGF mRNA have not been analyzed in detail in fibroblasts. In normal rat kidney fibroblasts, elevation of intracellular cAMP levels interfered with TGF-β-mediated induction of CTGF, whereas tyrosine kinase inhibition by herbimycin or activation of protein kinase C by phorbol ester was without effect (19).

LPA is generated by cleavage from membranes of stimulated cells. It is widely distributed in mammalian tissues and serum, reaching concentrations in the micromolar range (20). Cellular effects of LPA can be categorized as growth-related or cytoskeleton-dependent, resulting in the modulation of adhesion, chemotaxis, contraction, or aggregation (21). As a mitogen for fibroblasts and with additional effects on endothelial cells, macrophages, and vascular smooth muscle cells, LPA has been implicated in wound healing. Because of the role of CTGF in wound healing and fibrosis, it was tempting to speculate that, in addition to other early response genes such as c-fos and egr-1, LPA might induce CTGF. Recent results have demonstrated that, in mesangial cells, LPA is indeed able to induce CTGF expression (18). As a model system for the investigation of CTGF expression, we used an immortalized human renal fibroblast cell line exhibiting the major characteristics of nontransformed primary renal interstitial fibroblasts (22).

Materials and Methods
Materials
Recombinant human TGF-β was obtained from TEBU (Frankfurt, Germany). PD-98059 and SB-203580 were from Calbiochem (Bad Soden, Germany). LPA, forskolin, prostaglandin E1, and cytochalasin D were from Sigma (Deisenhofen, Germany). Pertussis toxin was from Biomol (Hamburg, Germany). Y-27632 was kindly provided by Yoshitomi Pharmaceutical Industries (Osaka, Japan). Toxin B from Clostridium difficile and C3 toxin from Clostridium limosum were kindly provided by Drs. F. Hofmann, H. Barth, and K. Aktories (Institute of Pharmacology, Freiburg University, Freiburg, Germany). C3 toxin was provided as a fusion protein with parts of CII toxin from Clostridium botulinum, to allow endocytotic uptake by cells (23). An antibody directed against human CTGF was kindly provided by FibroGen (South San Francisco, CA).

Cell Culture
Immortalized human renal fibroblasts were kindly provided by G. A. Müller (University of Göttingen, Göttingen, Germany) (22). The cells were grown in Dulbecco’s modified Eagle’s medium, supplemented with 2 mM L-glutamine, 4.5 g/L glucose, 100 U/ml penicillin, and 100 μg/ml streptomycin, with 10% FCS. Renal fibroblasts (0.8 to 1.0 × 10⁶ cells/10 ml) were plated in 100-mm Petri dishes in medium with 10% FCS. At subconfluence (after 2 to 3 d), cells were serum-starved for 1 d in Dulbecco’s modified Eagle’s medium containing 0.5% FCS.

Northern Blot Analysis
Northern blot analysis was performed as described previously (24). After stimulation for the indicated times, total RNA was extracted according to the protocol of Chomczynski and Sacchi (25), with minor alterations. The RNA yield was usually approximately 60 to 80 μg/10-cm Petri dish. Separation of total RNA (20 μg/lane) was achieved by using 1.2% agarose gels containing 1.9% formaldehyde, with 20 mM 3-(N-morpholino)propanesulfonic acid, 5 mM sodium acetate, 1 mM ethylenediaminetetraacetate, pH 7.0 as the gel running buffer. Separated RNA was transferred to nylon membranes by capillary blotting and was fixed by baking at 80°C for 1 to 2 h. The 18S and 28S rRNA forms were stained with methylene blue (0.04% in 500 mM sodium acetate, pH 5.2) and directly quantitated by densitometry.

Hybridization was performed with cDNA probes labeled with [32P]dCTP, using a NonaPrimer kit (Appligene, Heidelberg, Germany). A cDNA specific for human CTGF was obtained by reverse transcription-PCR amplification of the entire coding region of the gene and was cloned into the pTracer-CMV2 vector (Invitrogen BV, Groningen, The Netherlands). The primer sequences used for the amplification were 5’-GCCAACCATGACGCGCAG-3’ (sense) and 5’-TGCCATGTCTCCATACATCTTCTG-3’ (antisense).

Blots were prehybridized for at least 1 h at 40°C. The probes were allowed to bind overnight at the same temperature. Washing at 40°C was performed for 2 × 15 min under high-salt conditions [2 × SSC/0.2% sodium dodecyl sulfate (SDS)] and for 2 × 15 min under low-salt conditions (0.2 × SSC/0.2% SDS). DNA/RNA hybrids were detected by autoradiography using Kodak X-Omat AR film (Eastman Kodak, Rochester, NY). Quantitative analysis was performed by densitometric scanning of the autoradiographs (Bioprofil; Froebel, Wasserburg, Germany). All values were corrected for differences in RNA loading by calculation of the CTGF/18S RNA expression ratio.

Western Blot Analysis
Cellular proteins were isolated using RIPA buffer (50 mM Tris-HCl, pH 7.5, 1% (vol/vol) Triton X-100, 0.1% (wt/vol) deoxycholic acid, 0.1% (wt/vol) SDS, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium vanadate, 14 μM/ml aprotinin). For Western blot analysis, 50 to 100 μg of protein were separated by SDS-polyacrylamide gel electrophoresis (10% polyacrylamide) and transferred to a polyvinylidene fluoride membrane (Pall Biosupport Division, Dreieich, Germany). Blots were incubated in 5% skim milk, followed by overnight incubation with rabbit anti-human CTGF antibody. The membranes were then washed and incubated for 1 h with horseradish peroxidase-conjugated swine anti-rabbit IgG (DAKO, Glostrup, Denmark). Bound antibody was detected with the enhanced chemiluminescence reagent luminol (Autogen Bioclear UK, Wiltshire, UK).

Staining of Actin Filaments
Cells were cultured and growth-arrested on glass, eight-well, multiet slide (ICN, Cleveland, OH) placed in a Petri dish. Further treatments with different toxins and stimuli were performed in wet chambers. After treatment, cells were fixed with 3% paraformaldehyde in phosphate-buffered saline for 10 min and then permeabilized with 0.2% Triton X-100 in phosphate-buffered saline for 7 min at room temperature. For examination of DNA fragmentation, cells were incubated with Hoechst 33258 (8 μM/ml) for 5 min. After washing, the actin cytoskeleton was stained with rhodamine-phalloidin (Molecular Probes, Leiden, The Netherlands) for 20 min.
Results

Induction of CTGF by LPA in Renal Fibroblasts

Renal fibroblasts were serum-deprived by incubation overnight in medium with 0.5% serum and were then stimulated with LPA (1 to 30 μM) for 90 min (Figure 1A). The LPA-mediated upregulation of CTGF mRNA was concentration dependent, with a plateau at 10 to 20 μM LPA, i.e., concentrations that are detectable in serum (20). CTGF mRNA was upregulated transiently, with maximal levels being reached after 1 to 2 h. Thereafter, the steady-state levels of CTGF mRNA decreased (Figure 1B). TGF-β, which is the most potent inducer of CTGF in other types of fibroblasts (2), also induced CTGF mRNA in human renal fibroblasts, although with different kinetics (Figure 1C). Concomitantly with the increase in CTGF mRNA levels, stimulation of the fibroblasts with LPA led to time-dependent synthesis of CTGF protein, which was detectable in cellular homogenates, indicating close association of CTGF with the cells (Figure 1D). CTGF was detected as a double band at approximately 38 kD. No protein was detectable in the cell culture supernatants.

LPA binds to heptahelical receptors, which couple to different types of G proteins (21). Preincubation of the renal fibroblasts with pertussis toxin did not affect LPA-mediated induction of CTGF (Figure 2A). This finding suggested the involvement of G proteins of the Gq/11 or G12/13 type. The mitogen-activated protein (MAP) kinases p42/44 and p38 are signaling modules for Gi-mediated gene induction by LPA (26,27). Inhibition of p42/44 MAP kinase activation by PD-98059 and of p38 MAP kinase activity by SB-203580 did not significantly reduce the LPA-mediated induction of CTGF (Figure 2, B and C), indicating that neither pathway was essential for CTGF induction.

RhoA-Mediated Modulation of the Actin Cytoskeleton

LPA was demonstrated to activate the small GTPase RhoA via pertussis toxin-insensitive signaling (28). This activation was related to changes in cell morphologic features attributable to increased organization of the actin cytoskeleton (29). Cultured human renal fibroblasts demonstrated expression of actin stress fibers, which increased after treatment with LPA (Figure 3A). Inactivation of the GTPases Rac and Cdc42 by toxin B (30) completely disassembled the actin cytoskeleton within 1 h, comparably to cytochalasin D (which directly affects actin polymerization) (Figure 3A). No signs of apoptosis were detectable with nuclear DNA staining with Hoechst 33258 (data not shown). Specific inhibition of RhoA by C3 toxin disassembled the actin stress fibers, whereas the cortical actin fibers remained intact. The pattern of actin fibers in cells treated with C3 toxin was in accordance with the induction of lamellipodia with membrane actin ruffles by Rac and the induction of filopodia with actin microspikes by Cdc42; Rac and Cdc42 are not inactivated by C3 toxin (31). Resolution of actin stress fibers was also obtained when the Rho kinases, which are downstream targets of RhoA, were inhibited by Y-27632 (Figure 3A). Stimulation of the cells with LPA did not restore the actin cytoskeleton after disassembly by toxin B, cytochalasin D, or C3 toxin (data not shown). The alterations of the actin cytoskeleton were also visible as changes in cell morphologic features detected by light microscopy. As an example, contraction of the cells and development of a more spindle-like appearance after treatment with C3 toxin are shown in Figure 3B. The phenotype observed after treatment with C3 toxin was not reversed by subsequent treatment with LPA.

Figure 1. Lysophosphatidic acid (LPA) induction of connective tissue growth factor (CTGF) mRNA expression in human renal fibroblasts. (A) Concentration-dependent induction of CTGF mRNA. Cells were incubated with different concentrations of LPA for 90 min. CTGF mRNA expression was detected by Northern blot analysis. As a control for equal loading, methylene blue staining of 18S rRNA is also shown. Co, control. (B) Time course of CTGF mRNA elevation by LPA. Fibroblasts were treated with LPA (10 μM) for the times indicated. RNA was isolated and probed for CTGF and 18S rRNA. (C) Quantification of Northern blot results by densitometry. To correct for loading differences, CTGF mRNA expression was related to 18S rRNA expression. The expression of CTGF in control cells was set to 1, for comparison of different blots. Data are means ± SD of 10 (LPA), seven [transforming growth factor-β (TGF-β), 3 h], or 10 (TGF-β, 6 h) experiments. (D) Detection of CTGF protein in cellular homogenates by Western blot analysis. The blot is representative of three similar ones.
Involvement of RhoA in the LPA-Mediated Induction of CTGF mRNA

Interference with the activation of the Rho proteins RhoA, Rac, and Cdc42 by toxin B completely prevented LPA-mediated CTGF mRNA induction (Figure 4, A and D). Similarly, the basal expression of CTGF was abolished after treatment with toxin B. Baseline levels were barely affected by cytochalasin D, whereas destruction of the actin cytoskeleton by this compound prevented LPA-mediated induction of CTGF (Figure 4, A and D). Interference with CTGF mRNA induction was specifically related to inhibition of RhoA, as indicated by the strong inhibitory effect of C3 toxin, which specifically targets RhoA without effects on Rac or Cdc42 (Figure 4, B and D). Various proteins are involved in RhoA-mediated regulation of the actin cytoskeleton, including the Rho kinase family (32). Inhibition of these kinases by Y-27632 reduced CTGF expression, with maximal inhibition being observed with 5 to 10 μM Y-27632 (Figure 4, C and D). Apparent changes in basal CTGF mRNA expression did not prove to be statistically significant.
Regulatory Roles for RhoA and the Actin Cytoskeleton in TGF-β/Mediated Induction of CTGF

Disruption of the cytoskeleton by inhibition of the Rho kinases by Y-27632 or cytochalasin D also affected TGF-β-induced CTGF mRNA expression. When the cells were preincubated with either compound for 45 min and then stimulated with TGF-β for 6 h, induction of CTGF was almost completely prevented (Figure 5).

Reversal of cAMP-Mediated Changes in Cell Morphologic Features by LPA

Elevated levels of cAMP and subsequent activation of protein kinase A (PKA) also led to the disassembly of actin stress fibers in fibroblasts (33). Incubation of human renal fibroblasts with the cell-permeable cAMP analogue cBIMPs, which specifically activates PKA, or the adenylyl cyclase activator forskolin induced destruction of the actin fibers in <1 h (Figure 6A). Subsequent treatment with LPA completely restored the actin filaments. The disassembly of the actin cytoskeleton produced by elevated cAMP levels led to changes in cell shape, characterized by rounding of the cell bodies and the development of elongated processes. As an example, cells treated with forskolin are shown in Figure 6B. After treatment with LPA, the cells flattened and became indistinguishable from untreated control cells.
Interference with LPA-Mediated Induction of CTGF by PKA Activation

Incubation of the renal fibroblasts with forskolin or prostaglandin E₁, which also elevated intracellular cAMP levels by stimulating adenyl cyclase, reduced the basal expression of CTGF. LPA-mediated induction of CTGF was greatly impaired when the cells were preincubated with either forskolin or prostaglandin E₁ (Figure 7, A and C). Similar effects were observed when the cells were incubated with cell-permeable analogues of cAMP, i.e., 8-(4-chlorophenylthio)-cAMP or 8-(4-chlorophenylthio)-cAMP (Figure 7A).}

**Figure 6.** Morphologic changes of the cytoskeleton produced by activated protein kinase A (PKA). (A) Human renal fibroblasts, grown on glass slides, were treated with 5,6-dichlorobenzimidazole-1β-d-ribofuranosyl-3',5'-monophosphorothioate (cBIMPs) (250 μM) or forskolin (FK) (10 μM) for 45 min and were then incubated with 10 μM LPA for an additional 90 min. Control cells were left untreated (Co) or were treated with cBIMPs, forskolin, or LPA. Cells were fixed, and the actin cytoskeleton was stained with rhodamine-phalloidin. Arrows, examples of assembled and disassembled actin stress fibers. Photographs were taken under identical conditions. Original magnification, ×400. (B) The morphologic changes demonstrated in A were also visible by light microscopy, as shown for forskolin (FK)-treated cells. Photographs were taken under identical conditions. Original magnification, ×400.

**Figure 7.** Interference with LPA-stimulated CTGF mRNA expression by PKA activation. (A) Cells were preincubated with 10 μM forskolin (FK) or prostaglandin E₁ (PGE₁) for 45 min and were then stimulated with 10 μM LPA. After 90 min, cells were collected and RNA was isolated and probed for CTGF on a Northern blot. Methylene blue staining of 18S rRNA is also shown, to indicate equal gel loading. Co, control. (B) Cells were pretreated with cBIMPs (cB) (250 μM) or 8-(4-chlorophenylthio)-cAMP (CPT) (400 μM) for 45 min and were then stimulated with LPA (10 μM) for an additional 90 min. CTGF mRNA expression was detected by Northern blot analysis. (C) Northern blot results were quantitated by densitometry. The expression of LPA-stimulated cells was set to 100% (forskolin, n = 10; prostaglandin E₁, n = 3, means ± SD). *, P < 0.05; **, P < 0.005, compared with control cells and cells stimulated with LPA, respectively (two-sided t test for paired samples).
cBIMPs (34) (Figure 7, B and C). Again, LPA did not overcome the inhibitory effect of elevated cAMP levels.

**Discussion**

To date, TGF-β has been demonstrated to be the most potent inducer of CTGF expression in fibroblasts, with little effect of other growth factors such as platelet-derived growth factor or epidermal growth factor (summarized in Reference 2). We now demonstrate that activation of heptahelical receptors by LPA is a potent signaling pathway for the induction of CTGF in fibroblasts. LPA receptors couple to different types of trimeric G proteins. We previously demonstrated that induction of the early response genes egr-1 and cox-2 in mesangial cells was pertussis toxin-sensitive and is thus mediated by Gi proteins (27). Furthermore, LPA-mediated proliferation and migration of human skin fibroblasts were completely sensitive to pertussis toxin (35). CTGF induction, in contrast, was insensitive to pertussis toxin and is thus mediated by Gq/11 or G12/13 proteins. Both types of G proteins have been linked to LPA signaling (summarized in Reference 21). Activation of Gq/11 was linked to phospholipase C and p42/44 MAP kinase signaling. Inhibition of the activity of this MAP kinase by the specific inhibitor PD-98059 did not affect LPA-mediated CTGF induction. Therefore, it is unlikely that Gq/11 plays a major role in CTGF induction. LPA signaling via G12/13 is in line with the involvement of Rho proteins in CTGF induction; G12/13 proteins were identified as links between heptahelical receptors and the activation of the GTPase RhoA (36). LPA-mediated induction of CTGF was abrogated when the cells were pretreated with toxin B, a potent inhibitor of the three proteins of the Rho family (Rho, Rac, and Cdc42) (37). More specifically, induction was also impaired when RhoA was selectively inhibited by treatment of the cells with C3 exotoxin.

Rho proteins are involved in gene expression but are also prominent regulators of the actin cytoskeleton. Rho-associated serine/threonine kinase isozymes (Rho kinases) regulate the polymerization of stress fibers via inactivation of myosin light chain phosphatase (38). Inhibition of these kinases with the specific inhibitor Y-27632 (39) also impaired CTGF induction, suggesting a link between cytoskeletal integrity and the induction of CTGF. This was confirmed when the actin stress fibers were depolymerized by the direct action of cytochalasin D. Cytochalasin D impaired LPA-mediated CTGF induction to a similar extent, compared with C3 exotoxin. An important role of RhoA and the cytoskeleton in the regulation of CTGF was recently observed in mesangial cells, which in many other respects exhibit different signaling properties, compared with fibroblasts (18). As an example, induction of the early response gene cyclooxygenase 2 was positively regulated by the viral kinase pp60src in fibroblasts (40,41), whereas cyclooxygenase 2 was not inducible in v-src-transformed mesangial cells (42). Similarly, elevation of cAMP levels had no effect on cyclooxygenase 2 expression in mesangial cells (43) but induced cyclooxygenase 2 expression in fibroblasts (44). Regulation of CTGF by RhoA, in contrast, does not seem to be restricted to a specialized cell type.

Cytoskeletal changes were also observed when the intracellular levels of cAMP were elevated by cell-permeable cAMP analogues or by adenylyl cyclase activation by forskolin. A similar actin pattern was observed when the cells were treated with prostaglandin E1, suggesting activation of PKA via EP2 or EP4 receptors (45). The morphologic changes were reversed when the PKA-activated cells were subsequently treated with LPA; in <1 h, the cells rounded and the actin cytoskeleton reorganized into stress fibers. Interactions between cAMP-activated pathways and Rho-mediated structural changes have been observed in different cell types. In epithelial cells, activation of PKA caused rapid changes in cell morphologic features, which were attributable to inactivation of RhoA. Pretreatment of these cells with thrombin, which activated RhoA, prevented PKA-mediated morphologic changes (46). In these cells, the thrombin pathway dominated the cAMP pathway. The opposite was observed in PC12 cells, where elevated cAMP levels protected cells against LPA-mediated neurite retraction (47). The interaction between LPA and PKA seems to be dependent on the cell type, because LPA was able to reverse the morphologic changes caused by cAMP in astrocytes (48). Recombinant RhoA is a target of PKA, and phosphorylation of Ser-188 increased the interaction with the guanine nucleotide dissociation inhibitor, which translocates RhoA from the membrane to the cytosol (49). In line with this interpretation, elevated levels of cAMP resulted in enhanced cytosolic levels of RhoA and seemed to prevent membrane association (50). Activation of PKA thus results in inactivation of RhoA and may thus oppose the effects of RhoA activators such as LPA. The morphologic observations for renal fibroblasts are in agreement with the changes observed in astrocytes, because LPA was able to reverse the effects of cAMP in both cell types.

In contrast to the morphologic changes, however, LPA was not able to overcome the cAMP-mediated blockade of the signaling pathway leading to gene expression; LPA-mediated CTGF expression was inhibited by all compounds that elevated cAMP levels. If an equilibrium between phosphorylated and nonphosphorylated RhoA is assumed, then LPA might be able to shift the equilibrium to the nonphosphorylated form, thus reversing the morphologic effects. Because nothing is known regarding the phosphatases mediating RhoA dephosphorylation or the kinetics of those reactions, it might be speculated that the transition is too slow to allow the rapid signaling that induces CTGF mRNA expression. Furthermore, we cannot exclude additional, RhoA-independent mechanisms by which PKA might interfere with LPA-mediated CTGF expression.

The regulatory role of RhoA is not unique for LPA signaling. TGF-β-mediated induction of CTGF was strongly reduced when RhoA signaling was inhibited by a Rho kinase inhibitor or when the actin cytoskeleton was directly disassembled by cytochalasin D. These data are in agreement with data published previously; in H-ras-transformed fibroblasts, inhibition of RhoA by C3 exotoxin abrogated the ability of TGF-β to induce actin stress fibers (51). Furthermore, elevated levels of cAMP blocked TGF-β-induced changes in cell morphologic features and CTGF expression in normal rat kidney fibroblasts (19). Recent data obtained in a different cell type, *i.e.*, renal
mesangial cells, also indicated that inhibition of Rho proteins interfered with TGF-β-mediated CTGF induction (18). Therefore, the regulatory roles of RhoA and the cytoskeleton are not restricted to a specific stimulus or cell type but seem to represent more general features of CTGF regulation.

Given the role of CTGF in matrix synthesis and its proposed role in the development of fibrosis, targeting of RhoA might be a way to interfere with these processes. Rho proteins are inactivated by various bacterial toxins, as used in this study. At the pharmacologic level, hydroxymethyl glutaryl-CoA reductase inhibitors might be more appropriate. In addition to their lipid-lowering effects, these compounds interfere with the isoprenylation, and thus the activation, of Rho proteins (52). Preliminary data indicate that lovastatin and simvastatin, two clinically used statins, indeed interfere with CTGF synthesis.

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References


Regulation of CTGF by LPA, RhoA, and cAMP 1861