Induction of Connective Tissue Growth Factor by Activation of Heptahelical Receptors

MODULATION BY Rho PROTEINS AND THE ACTIN CYTOSKELETON*

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Expression of connective tissue growth factor (CTGF) was induced in renal mesangial cells by activation of heptahelical receptors by serotonin (5-HT) and lysophosphatidic acid (LPA). Induction of CTGF mRNA was transient with maximal expression after 1 to 2 h, whereas induction of CTGF by transforming growth factor beta (TGF-β) increased over time. In contrast to the induction of other early response genes (Egr-1 and cyclooxygenase-2), LPA-mediated induction of CTGF was p38 mitogen-activated protein kinase-sensitive and independent of p42/44 MAP kinase activation. 5-HT-mediated CTGF induction was due to activation of 5-HT_1A receptors and likewise independent of p42/44 MAP kinase activation. Upon stimulation, enhanced levels of CTGF protein were detected in cellular homogenates, whereas no protein was detectable in cell culture supernatants. Inhibition of proteins of the Rho family by toxin B abrogated basal as well as CTGF expression stimulated by LPA, 5-HT, and TGF-β. Inhibition of the downstream mediator of RhoA, the Rho kinase by Y-27632 partially reduced induction of CTGF by LPA and TGF-β. Toxin B not only affected gene expression, but disrupted the actin cytoskeleton similarly as observed after treatment with cytochalasin D. Disassembly of actin stress fibers by cytochalasin D partially reduced basal and stimulated CTGF expression. These data indicate that an intact actin cytoskeleton is critical for the expression of CTGF. Elimination of the input of Rho proteins by toxin B, however, was significantly more effective and their effect on CTGF expression thus goes beyond disruption of the cytoskeleton. These findings thus establish activation of heptahelical receptors coupled to pertussis toxin-insensitive G proteins as a novel signaling pathway to induce CTGF. Proteins of the Rho family and an intact cytoskeleton were identified as critical determinants of CTGF expression induced by LPA and 5-HT, and also by TGF-β.

Connective tissue growth factor (CTGF) belongs to the family of low affinity insulin-like growth factor binding proteins, which consists of Mac25, the nov oncogenes, and cyr61 (1), and is also classified as a member of the CCN (CYR61, CTGF, and NOV) family (2, 3). These proteins share structural homologies and function as growth modulators. CTGF was first purified from conditioned medium of human umbilical vein endothelial cells and shown to account for much of the bioactivity previously attributed to platelet-derived growth factor (4). Recent data obtained with aortic smooth muscle cells and breast cancer cells demonstrated that CTGF may act as a mediator of growth arrest and apoptosis (5–7). In fibroblasts, it is most potently induced by transforming growth factor beta (TGF-β) (8). It stimulates fibroblast cell proliferation and mediates TGF-β-induced anchorage-independent growth (9). Furthermore, CTGF is a potent stimulator of extracellular matrix synthesis (10, 11).

Elevated levels of CTGF are found in fibrotic lesions (e.g. Refs. 12–14) and 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 (15). In the glomerulus, basal expression of CTGF was detected in epithelial podocytes. In the inflamed glomerulus, CTGF was up-regulated in proliferating epithelial cells and also observed in mesangial cells. Mesangial cells cultured in vitro express basal levels of CTGF mRNA, which are further increased by TGF-β (16). In accordance with elevated CTGF expression in diabetic glomerulosclerosis (15, 16), elevation of glucose levels enhanced CTGF mRNA levels in cultured mesangial cells (5). Up-regulation of CTGF by glucose was blocked by anti-TGF-β antibodies, confirming CTGF as a downstream target of TGF-β in mesangial cells (16). Besides TGF-β, CTGF itself was able to induce its own mRNA expression (16). Mesangial cells are thus target cells of CTGF, as also shown by the induction of extracellular matrix proteins (fibronectin and collagen I and IV) (5). CTGF belongs to the group of proteins coded for by immediate early response genes, which in general are induced by a variety of different mediators. As an example, cyclooxygenase-2 (Cox-2) is induced by growth factors, cytokines, and low molecular mediators acting via serpentine receptors (17). This prompted us to further investigate the regulation of CTGF mRNA and protein expression in renal mesangial cells.

Lysophosphatidic acid (LPA) is generated by cleavage of glycerophospholipids in membranes of stimulated cells. Increased release of LPA is observed in tissue injury, inflammation, and neoplasia (18). Activated platelets are an abundant source of LPA, and high levels of the lysophospholipid (2–20 μM) are detectable in serum (19). Via binding to seven-trans-

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The abbreviations used are: CTGF, connective tissue growth factor; Cox, cyclooxygenase; LPA, lysophosphatidic acid; MAP kinase, mitogen-activated protein kinase; 5-HT, serotonin; TGF-β, transforming growth factor β; p38, pertussis toxin; FCS, fetal calf serum; MOPS, 4-morpholinepropanesulfonic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
membrane receptors (edg receptors), LPA modulates cell proliferation and differentiation and mediates cellular effects such as chemotaxis, adhesion, contraction, or aggregation, which are related to cytoskeletal rearrangements (18). Treatment of mesangial cells with LPA led to contraction of the cells (20) and stimulated proliferation (21, 22). Proliferation was shown to be mediated by the induction of the expression of the immediate early gene Egr-1 (23). Likewise, Cox-2, another example of an early response gene, was rapidly induced by LPA in mesangial cells (23). LPA-mediated induction of the early response genes was pertussis toxin-sensitive, i.e. mediated by G proteins of the G type. Furthermore, activation of heptahelical receptors coupling to pertussis toxin-insensitive G proteins also led to the induction of these early response genes as exemplified by serotonin (5-HT) (24, 25). Activation of p42/44 MAP kinase was a common signaling module in both pathways: the kinase was rapidly activated by LPA or 5-HT, and inhibition of p42/44 MAP kinase prevented induction of Cox-2 or Egr-1 (23, 26). It was thus tempting to speculate that the early response gene CTGF might be another target of LPA and/or 5-HT in mesangial cells. Based on the previous studies on the induction of Egr-1 and Cox-2, rat mesangial cells were used to investigate the induction of CTGF by activation of heptahelical receptors and to delineate the signaling pathways responsible for CTGF induction, which have not yet been described in detail in any cell type.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human TGF-β was obtained from TEBU, Frankfurt, Germany. PD-98059 was from Calbiochem, Bad Soden, Germany. LPA, serotonin (5-HT), and cytochalasin D were from Sigma, Deisenhofen, Germany. Pertussis toxin (PTX) was from Biomol, Hamburg, Germany. Cell culture reagents were from Biochrom, Berlin, Germany. FCS was from Life Technologies, Inc., Piggenstein, Germany. Toxin B from Clostridium difficile was kindly provided by Drs. F. Hofmann and K. Aktories, Freiburg, Germany. Y-27632 was kindly provided by Yoshitomi Pharmaceutical Industries, Osaka, Japan.

Cell Culture—Rat mesangial cells were isolated as described (27) and were grown in Dulbecco’s modified Eagle’s medium supplemented with 2 mM-l-glutamine, 5 µg/ml insulin, 4.5 g/liter glucose, 100 units/ml penicillin, and 100 µg/ml streptomycin containing 10% FCS. Mesangial cells (0.5–1.0 × 10⁶ cells/10 ml) were plated in 100-mm Petri dishes in medium with 10% FCS. At subconfluency (after 3–4 days) cells were serum-starved in Dulbecco’s modified Eagle’s medium containing 0.5% FCS for 2–3 days.

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 (28) with minor alterations. Usually, RNA yield was about 30–40 µg/10-cm Petri dish. Separation of total RNA (10 µg/lane) was achieved by use of 1.2% agarose gels containing 1.9% formaldehyde with 1× MOPS as gel running buffer. Separated RNA was transferred to nylon membranes by capillary blotting and fixed by baking at 80 °C for 2 h.

Hybridization was performed with cDNA probes labeled with [³²P]dCTP using the NonaPrimer kit from Appligene, Heidelberg, Germany. The specific Cox-2 probe was a 1.156-kilobase EcoRI fragment from the 5′-end of mouse cDNA (29). A cDNA specific for CTGF (full-length cDNA of human CTGF) was kindly provided by N. Wahab, London, UK (30). The GAPDH (glyceraldehyde-3-phosphate dehydrogenase) probe was obtained with a 500-base pair reverse-transcribed fragment. DNA/RNA hybrids were detected by autoradiography using Kodak X-Omat AR film. As a control for equal loading of the gels, the housekeeping gene GAPDH or the 18 S rRNA was hybridized or the blotted 18 S rRNA was stained with methylene blue (0.04% in 500 mM sodium acetate, pH 5.2) and directly quantitated by densitometry.

Quantitative analysis of the autoradiographs was performed by densitometric scanning (Froebel, Wasserburg, Germany). All values were corrected for differences in RNA loading by calculating the ratio of the specific bands to GAPDH or 18 S rRNA expression. The two-sided Student’s t test for paired samples was used to calculate significant differences.

Western Blot Analysis—Cellular proteins were isolated using radio-

![Fig. 1. Induction of CTGF mRNA. A, mesangial cells were treated with medium (C) or stimulated with LPA (L, 10 µm), serotonin (S, 1 µm), or TGF-β1 (T, 5 ng/ml) for the times indicated. CTGF mRNA expression was detected by Northern blot analysis. B, mesangial cells were incubated with LPA (10 µm) for the times indicated. As a control for equal loading of the gels, the ratio of CTGF expression and 18 S rRNA or GAPDH was used. To compare the mRNA expression of different experiments, expression of control cells was set to 0 and expression after 60 min of stimulation was set to 100%. Data are means ± S.D. of five experiments. C, mesangial cells were incubated with different concentrations of LPA for 2 h. CTGF expression after stimulation with 25 µM LPA was set to 100%. Data are means ± half range of two experiments. D, mesangial cells were incubated with different concentrations of LPA in the presence or absence of 5 ng/ml TGF-β for 2 h. E, mesangial cells were preincubated with cycloheximide (CHX, 10 µg/ml) for 3 h and then analyzed (3 h). They were further incubated with or without LPA (10 µm) or CHX as indicated for 90 min (3 h + 90 min).](http://www.jbc.org/)

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immune precipitation buffer (50 mM Tris/HCl, pH 7.5, 1% (v/v) Triton X-100, 0.1% (w/v) deoxycholic acid, 0.1% (w/v) SDS, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium vanadate, 14 μg/ml apro- 
tinin). For Western blot analysis, 75 μg of protein was separated by SDS-polyacrylamide gel electrophoresis (10% polyacrylamide), trans- 
ferred onto a polyvinylidene difluoride membrane (Pall Biosupport Di-
vision, Dreieich, Germany) and probed with an antibody directed against mouse CTGF. The antibody was kindly provided by S. Werner, 
Zurich, Switzerland (31).

Staining of Actin Filaments—Cells were cultured and growth-ar- 
rested on glass 8-well multitest slides (ICN, Cleveland, OH) placed in a 
Petri dish. After stimulation, the cells were fixed with 3% paraform- 
aldehyde 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. After washing, the actin cytoskeleton was stained with rhodamine-phalloidin complex (Molecular Probes, Leiden, The Nether-
lands) for 20 min.

RESULTS

Induction of CTGF mRNA Expression by Activation of Hep-
thelial Transmembrane Receptors—TGF-β has been charac-
terized as a potent inducer of CTGF in different cell types, 
among them human mesangial cells (5). This was confirmed 
when rat mesangial cells were treated with TGF-β with subse-
quent analysis of CTGF mRNA expression (Fig. 1A). A 2-fold 
stimulation was observed after 2 h (1.8 ± 0.4, n = 3, means ± S.D.). CTGF mRNA levels were further increased at later time 
points (4 h, 3.7 ± 0.8, n = 4, means ± S.D., p < 0.005). Two activators of heptahelical receptors, serotonin (5-HT) and lys-
ophosphatidic acid (LPA) showed a similar but more transient 
response. Maximal stimulation of CTGF by LPA was observed 
after 1 h (Fig. 1B). Stimulation was 2.0 ± 0.4-fold (means ± S.D., n = 9, p < 0.05) with 10 μM LPA. A similar stimulation 
was observed with 5-HT (1.9 ± 0.7, n = 7, means ± S.D., p < 
0.05, stimulation time 2 h). Induction of CTGF by LPA was 
concentration-dependent. Micromolar concentrations were nec-
sary and sufficient to induce CTGF mRNA induction (Fig. 
1C). Increased proliferation of mesangial cells and the induc-
tion of the early response genes Egr-1 and Cox-2 were observed 
in the same concentration range of LPA (23). LPA concentra-
tions were within the range reported to occur in serum (2–20 
μM (19)). Higher concentrations were not used to avoid nonspe-
cific effects of the lysophospholipid. When mesangial cells were 
incubated with LPA plus TGF-β, the increase in CTGF mRNA 
expression was additive (Fig. 1D). Low concentrations of LPA, 
which by themselves did not induce CTGF expression, did not
significantly augment TGF-β-mediated induction of CTGF. Additivity was also observed with TGF-β and 5-HT (data not shown). Treatment of mesangial cells with the inhibitor of protein synthesis cycloheximide revealed the dynamics of basal CTGF protein synthesis cycloheximide was set to 100%. Data are means of three experiments.

**FIG. 4. MAP kinase-independent induction of CTGF.** A, mesangial cells were preincubated with PD-98059 (10 and 20 μm, PD10 and PD20) for 30 min. Then the cells were further incubated with LPA (10 μM) for 1 h. Northern blot analysis was used to detect CTGF and Cox-2 mRNA expression. B, mesangial cells were preincubated with PD-98059 as indicated (PD 10 μM) for 30 min and then further incubated with LPA (10 μM) or 5-HT (1 μM) for 1 h. To compare different experiments, expression of CTGF after stimulation with LPA and 5-HT, respectively, was set to 100%. Data are means of three experiments.

**FIG. 5. Involvement of Rho proteins in CTGF regulation.** A, mesangial cells were preincubated with toxin B for 3 h at the concentrations indicated. Then the cells were stimulated with LPA (10 μM) for 1 h. The blot is representative of four experiments with similar results. B, mesangial cells were pretreated with toxin B for 3 h at the concentrations indicated. Then the cells were stimulated with 5-HT (1 μM) for 1 h. Expression of CTGF and Cox-2 mRNA was detected by Northern blot analysis. The blot is representative of three experiments with similar results. C, mesangial cells were preincubated with Y-27632 (10 μM) for 1 h and then stimulated with LPA (L, 10 μM) for 1 h and with TGF-β (T, 5 ng/ml) for 4 h D, mesangial cells were preincubated with toxin B (ToxB, 5 ng/ml) for 1 h and then stimulated with LPA plus TGF-β (L/T, 10 μM and 5 ng/ml) for 3 h. CTGF protein expression was detected in cellular homogenates by Western blot analysis.

**Differential Signaling Pathways for the Induction of CTGF and Cox-2**—As described before (23) expression of LPA-mediated Cox-2 mRNA was strongly reduced by pretreatment of the cells with pertussis toxin (PTX), whereas 5-HT-mediated induction of this early response gene was not affected by PTX, indicating coupling to different types of G proteins (Fig. 3A). Preincubation with PTX for 18 h did not significantly affect CTGF induction by LPA or 5-HT, indicating predominant activation of G proteins of the G 12/13 family in both signaling pathways (Fig. 3, A and B). In line with these results, 5-HT-mediated induction of CTGF was prevented by preincubation with ketanserin, a specific inhibitor of 5-HT2A receptors, which couple to Gαs proteins (Fig. 3C). Co-incubation of mesangial cells with 5-HT and LPA did not further enhance CTGF expression, whereas Cox-2 expression was increased (Fig. 3A and Ref. 23).

LPA and 5-HT, both activate p42/44 mitogen-activated protein (MAP) kinases in mesangial cells (26, 32). These kinases were shown to be essential parts of LPA- and 5-HT-mediated induction of the early response genes Egr-1 and Cox-2 in mesangial cells (Fig. 4A and Refs. 23 and 25). Treatment of me-
sangial cells with the MEK inhibitor PD-98059 led to a concentration-dependent inhibition of MAP kinase activity reaching over 90% inhibition at a concentration of 20 μM (32). The basal expression of CTGF was reduced by about 10% when the cells were incubated with 10 or 20 μM PD-98059 (Fig. 4, A and B). Induction of CTGF by LPA or 5-HT, however, was not impaired by PD-98059 and, thus, was independent of p42/44 MAP kinase activation.

Role of Rho Proteins in CTGF Induction—Rho proteins have been characterized as downstream mediators of LPA signaling in many cellular systems (e.g. Refs. 33 and 34). Treatment of mesangial cells for 3 h with various concentrations of toxin B, an inhibitor of RhoA, Rac1, and Cdc42, led to a concentration-dependent inhibition of both basal and LPA-induced CTGF expression (Fig. 5A). Reduction of CTGF levels by 10 ng/ml toxin B was complete, and no expression was detectable even at longer exposure times of the blot membranes (not shown). Likewise, 5-HT-mediated induction of CTGF was sensitive to treatment with toxin B (Fig. 5B). Induction of the early response gene Cox-2 was also reduced but to a lesser extent (Fig. 5, A and B). Involvement of RhoA in LPA-mediated signaling was shown by the inhibitor Y-27632, which specifically interferes with Rho kinase, a downstream target of RhoA (35). The inhibitor reduced LPA-mediated induction of CTGF by about 50% (51.8 ± 16.0, means ± S.D., n = 3, p < 0.05; Fig. 5C). It also interfered with TGF-β signaling, as did toxin B (see below). Inhibition of CTGF expression by toxin B was also observed at the protein level (Fig. 5D).

Role of the Actin Cytoskeleton in CTGF Induction—Inhibition of Rho family proteins strongly affected the actin cytoskeleton. Mesangial cells in culture show a high degree of actin filaments organized in stress fibers (Fig. 6). Treatment of mesangial cells with toxin B led to time-dependent changes in morphology due to a disorganization of the actin cytoskeleton. A more rapid destruction of the stress fibers was observed when the cells were treated with cytochalasin D. Cytoskeletal rearrangement as a possible explanation for the effect of toxin B on CTGF mRNA expression was investigated by comparison of toxin B and cytochalasin D. Pretreatment of mesangial cells for 1 h with toxin B (5 ng/ml) completely inhibited basal and TGF-β- or LPA-mediated induction of CTGF, whereas treatment with cytochalasin (1 μg/ml) partially reduced basal and stimulated CTGF induction (Fig. 7). The effect of cytochalasin D was concentration-dependent, 0.5 μg/ml being less effective than 1 μg/ml, with no further inhibition of CTGF expression when the concentration of cytochalasin D was increased from 1 to 2 μg/ml (data not shown).

DISCUSSION

The low molecular weight mediators LPA and 5-HT regulate mesangial cell contraction, proliferation, and gene induction and thus play a role in the control of glomerular hemodynamics and the progression of glomerular nephritis (36, 37). Furthermore, 5-HT has been related to increased matrix production in mesangial cells by induction of TGF-β and enhanced synthesis of type IV collagen (38). Induction of CTGF by LPA and 5-HT in mesangial cells further relates these mediators to the development and progression of renal fibrosis.

In mesangial cells, CTGF has primarily been characterized as a downstream mediator of TGF-β, but was also induced in an autocrine manner by recombinant CTGF (16). The data presented characterize activation of pertussis toxin-insensitive heptahelical receptors by LPA and 5-HT as novel signaling pathway to mediate CTGF induction. Induction was transient with maximal mRNA levels reached after 1 to 2 h. Similar kinetics were observed recently, when fibroblasts were stimulated by factor VIIa and thrombin (39), whereas des-Arg10-kallidin augmented CTGF mRNA levels more slowly, due to message stabilization (40). Activation of heptahelical receptors may thus differentially affect CTGF expression, possibly dependent on the cell type or the coupling to different downstream signaling pathways. CTGF induction by LPA or 5-HT was insensitive to pertussis toxin, suggesting involvement of G proteins of the Gα11 or Gα13 family. Regarding pertussis toxin-insensitive G proteins, LPA receptors seem to couple primarily to G12/13 proteins (e.g. Refs. 41 or 42 and citations therein), suggesting that this type of G protein might also be involved in LPA-mediated induction of CTGF. 5-HT2A receptors have been
characterized on mesangial cells to mediate the mitogenic effects of 5-HT as well as induction of immediate early response genes (25, 43, 44). Consistently, these effects were pertussis toxin-insensitive in line with coupling of 5-HT2A receptors to G_{i/1} proteins. In contrast to the induction of CTGF, LPA-mediated induction of the transient expression of early response genes Egr-1 or Cox-2 was pertussis toxin-sensitive in mesangial cells (23) as was c-fos induction in fibroblasts (45), indicating involvement of G proteins of the G_{i} type. LPA receptors have not yet been characterized in mesangial cells, and it is thus not clear whether pertussis toxin-sensitive and -insensitive effects are mediated by different receptors or by differential coupling of G proteins.

Previous studies have shown activation of p42/44 MAP kinases in mesangial cells within 2 min by LPA, which was sensitive to pertussis toxin, whereas activation by 5-HT was pertussis toxin-insensitive (32). Interference with p42/44 MAP kinase activation led to an almost complete inhibition of Cox-2 and Egr-1 expression (23, 25). Activation of p42/44 MAP kinases did not contribute significantly to LPA- or 5-HT-mediated CTGF expression, consistent with signaling pathways different from those leading to Cox-2 or Egr-1 expression. Induction of Cyr61, a protein closely related to CTGF, has recently been reported to be differentially sensitive to PD-98059 inhibition depending on the stimulus used (39). Whether p42/44 MAP kinase may also be involved in CTGF induction under certain conditions remains to be investigated.

The small GTP-binding protein RhoA is a downstream signaling molecule of LPA in many cell types (e.g. Refs. 33 and 34). Inhibition of Rho proteins by toxin B resulted in a concentration-dependent complete suppression of CTGF mRNA expression. This effect was not restricted to LPA-mediated CTGF expression but was also observed when the cells were stimulated with 5-HT or TGF-β. Compared with Cox-2 or Egr-1 mRNA expression, induction of CTGF was particularly sensitive to toxin B treatment, possibly related to the different signaling pathways activated. Basal expression of CTGF, which was dependent on continuous transcriptional activity, as shown by the inducing effect of cycloheximide, was reduced to a similar extent.

Concomitantly with the inhibition of CTGF mRNA expression, toxin B disrupted the actin cytoskeleton. Actin stress fibers, which are strongly expressed in mesangial cells cultured in vitro, were first dissolved and later appeared in a condensed form around the nucleus. Previous studies had shown that treatment with toxin B in the concentrations used did not lead to cell death by apoptosis or necrosis (23). Inhibition of Rho kinase, a downstream kinase of RhoA, implicated in RhoA-mediated actin polymerization (46), also impaired LPA-mediated CTGF expression, hinting to a role for RhoA and stress fiber organization in CTGF induction. Direct disruption of the cytoskeleton by cytochalasin D also strongly affected the induction of CTGF by LPA and even more profoundly by TGF-β. Compared with Cox-2 or Egr-1 mRNA expression, induction of CTGF was particularly sensitive to toxin B treatment, possibly related to the different signaling pathways activated. Basal expression of CTGF, which was dependent on continuous transcriptional activity, as shown by the inducing effect of cycloheximide, was reduced to a similar extent.

CTGF is a secreted protein, but due to its strong binding to heparin and other matrix components, it is detectable in the supernatants or as cell-associated protein depending on the cell type investigated (48, 49). In a recent paper, Riser et al. (16) observed increased levels of CTGF protein in mesangial cell supernatants after treatment of the cells with heparin, in accordance with a high portion of cell-associated CTGF. These data are in accordance with our results, where CTGF protein was only detectable in cellular homogenates. In accordance with the increase in steady-state levels of CTGF mRNA, stimulation of mesangial cells with TGF-β or LPA also time-dependently increased CTGF protein. A single regulated band corresponding to the one brought about by toxin B, indicating specific effects of interference with Rho protein activation. Multiple target proteins of RhoA and the other members of the family, the involvement of which in CTGF mRNA expression is not excluded, have been described in different cell types, but mediators leading to gene expression have not yet been identified (34).

CTGF is therefore likely to be a downstream mediator of LPA and 5-HT signaling, playing a role in the regulation of mesangial cell proliferation and matrix deposition in the glomerulus.
respect to the detection of proteolytic and potentially active CTGF fragments (50).

Taken together, induction of CTGF mRNA and protein in mesangial cells is not restricted to TGF-β as a stimulus but is stimulated by activation of heptahedral receptors coupled to pertussis toxin-insensitive G proteins. In the present study we showed activation by serotonin and LPA, but other activators of heptahedral receptors might also turn out to be regulators of CTGF expression thus extending the biological context of CTGF activation. The strong impact of the cytoskeletal organization on CTGF deserves further attention, because mesangial cells are contractile cells that change their phenotype during glomerular injury.

The involvement of RhoA in the regulation of CTGF bears pathophysiological and pharmacologically relevant implications: RhoA signaling is modulated by cGMP- and cAMP-dependent kinases (51–53), thus linking regulation of CTGF expression to activators of these pathways such as nitric oxide or activators of adenylyl cyclase. By interference with isoprenylation, the activity of Rho proteins is inhibited by 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors (statins) (54), thus providing another rationale for their use in experimental and clinical settings (55). Supported by S. Werner, Zurich, Switzerland, the cDNA directed against CTGF by N. Wahab, London, UK, and toxin B by Drs. F. Hofmann and K. Aktories, Freiburg, Germany.

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