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The Rho-kinase pathway regulates angiotensin II-induced renal damage

MÓNICA RUPÉREZ, ELSA SÁNCHEZ-LÓPEZ, LUIS MIGUEL BLANCO-COLIO, VANESA ESTEBAN, JUAN RODRÍGUEZ-VITA, JUAN JOSÉ PLAZA, JESÚS EGIDO, and MARTA RUIZ-ORTEGA

Vascular and Renal Research Laboratory, Fundación Jiménez Diaz, Universidad Autónoma, Madrid, Spain

The Rho-kinase pathway regulates angiotensin II-induced renal damage.

Background. Angiotensin II (AngII) is a key factor in the pathogenesis of renal damage. AngII via AngII type 1 receptors activates several intracellular signaling systems, including the small guanosine triphosphatase Rho and its downstream effector Rho-dependent serine-threonine kinase (Rho-kinase). The Rho/Rho-kinase pathway contributes to inflammatory and proliferative changes observed in cardiovascular diseases. However, the data on renal diseases are scarce. The aim of this study was to investigate the effect of Rho-kinase inhibition in AngII-induced renal damage.

Methods. We used the model of systemic AngII infusion into normal rats (100 ng/kg per minute; subcutaneous osmotic minipumps), and some animals were treated with the Rho-kinase inhibitor Y-27632 (30 mg/kg per day). In the kidneys of these animals, we evaluated renal lesions, transcription factor activity (by electrophoretic mobility shift assay), and messenger RNA (by polymerase chain reaction) and protein expression levels (by Western blot and/or immunohistochemistry) of proinflammatory and profibrotic factors.

Results. Rats infused with AngII for three days present renal inflammatory cell infiltration and slight tubular damage, which were diminished by treatment with the Rho-kinase inhibitor Y-27632. AngII activates nuclear factor-κB and causes overexpression of proinflammatory factors, including cytokines (tumor necrosis factor α) and chemokines (monocyte chemotactic protein-1), and of profibrotic factors (connective tissue growth factor). Treatment of AngII-infused rats with Y-27632 decreases the upregulation of these proinflammatory and profibrotic mediators.

Conclusion. These data demonstrate that the Rho-kinase pathway is involved in renal damage caused by AngII through the regulation of proinflammatory and profibrotic mediators. These results suggest that inhibition of the Rho-kinase pathway represents a novel therapy for renal diseases associated with local AngII generation.

Angiotensin II (AngII), the main peptide of the renin-angiotensin system, has been implicated in the pathogenesis of renal diseases [1, 2]. Renin-angiotensin system blockers are commonly used in the treatment of hypertension and proteinuria in humans [3]. In experimental models of renal injury, pharmacologic blockade of AngII actions, by angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor (AT) antagonists, ameliorates renal damage and decreases proteinuria [1–3]. Many studies have investigated the molecular mechanisms involved in AngII-induced renal damage. In cultured renal cells, AngII causes hypertrophy or proliferation, depending on the cell type, overexpression of proinflammatory factors, and accumulation of extracellular matrix proteins [1, 2, 4]. Systemic infusion of AngII causes renal damage [5–9] through the activation of intracellular signaling systems and the regulation of several mediators. We have observed that AngII increases renal activity of the nuclear factor-κB (NF-κB) and upregulates NF-κB-related cytokines and chemokines, including tumor necrosis factor-α (TNF-α) and monocytic chemotactic protein-1 (MCP-1), correlated with the presence of inflammatory cells [7, 8]. In addition, in AngII-infused rats, overexpression of profibrotic factors, such as the connective tissue growth factor (CTGF), was observed in the kidney and in the aorta in association with extracellular matrix accumulation [9, 10].

AngII acts through two receptor subtypes, AT_1 and AT_2. The AT_1 are G-coupled receptors and activate small G proteins, including Ras, Rac1, RhoA, and the Rho-kinase system [11, 12]. Recently, the Rho family of small guanosine triphosphatase (GTPase) has been reported as playing an important role in AngII signaling, including actin reorganization, vasoconstriction, hypertrophy, and gene expression [12–15]. The Rho family of GTP-binding proteins, which includes Rho, Rac, and Cdc42, among 30 proteins, has well-documented effects on cellular contraction, proliferation, actin cytoskeletal organization, and regulation of gene transcription [16]. Rho GTPases act as molecular switches driving upstream signals to a plethora of downstream effector pathways. Several studies suggest that Rho-kinase, a downstream effector molecule of Rho [17], plays an important role for various cellular functions, including focal adhesions, motility, contraction, and cytokinesis [18–21]. In hypertensive rats, the Rho-kinase

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system in blood vessels is activated and contributes to the pathogenesis of hypertensive vascular disease [22]. Rho has recently been implicated in the etiology of renal fibrosis, and it seems to be necessary for transforming growth factor β (TGF-β)-induced upregulation of CTGF [23]. The Rho-kinase inhibitor Y-27632 reduces tubulointerstitial fibrosis in a mouse model of unilateral ureteral obstruction [24]. Thus, Rho signaling pathways could be regarded as a novel therapeutic target for hypertension and renal damage. Strategies designed to target this molecule have been successful in animal models, and some are currently in clinical trials [25].

Our aim was to investigate whether the Rho/Rho-kinase pathway could modulate some in vivo AngII responses in the kidney. For these studies, we evaluated the effect of the Rho-kinase inhibitor Y-27632, a selective inhibitor of the serine/threonine Rho-kinases I and II that is competitive for their adenosine triphosphate-binding site [26, 27], in renal damage caused by systemic infusion of AngII, and studied the effect on the regulation of proinflammatory and profibrotic factors.

METHODS

Materials

The selective inhibitor of Rho-associated kinase Y-27632 was from TOCRIS Cookson (Bristol, UK). Rabbit anti-CTGF antibody was obtained from Torrey Pines Biolabs (Houston, TX, USA) [9], anti-tubulin from Sigma (St. Louis, MO, USA), and secondary antibodies from Amersham Biosciences (Piscataway, NJ, USA).

Experimental studies

AngII was systemically infused into female Wistar rats (subcutaneously by osmotic minipumps, Alza Corp., Mountain View, CA, USA), at a dose of 100 ng/kg per minute. To determine the effect of Rho-kinase activation, a group of rats was treated daily with the Rho-kinase inhibitor Y-27632 (30 mg/kg per day, intraperitoneally), starting 24 hours before AngII-infusion. Animals were sacrificed at three days ($N = 7$ rats each group). The kidneys were immediately removed, a part was frozen for RNA and protein studies, and the rest was fixed and paraffin-embedded [9]. Control groups of animals infused with saline solution with or without treatments were also studied. Animals were handled following European guidelines.

Gene and protein studies

Total RNA was isolated with Trizol (Gibco, Rockville, MD, USA), and gene expression was analyzed by reverse-transcriptase polymerase chain reaction [8]. The specific primers used were: TNFα (sense: 5′-ATGAGCACAGAAAGCATGATCC -3′, antisense: 5′-GAAGATGATCTGAGTGTG -3′), MCP-1 (sense: 5′-TTCTGGGCCTGTTGTTCACA-3′, antisense: 5′-GGTCACTTCTACAGAAGTCC-3′) [28], and G3PDH (sense: 5′-AATGCATCCTGCACCACCAA-3′, antisense: 5′-GTAGCCATATTCATTGTCATA-3′), which yield a 251 bp, 409 bp, and 515 bp product, and polymerase chain reaction (PCR) (1 minute at 94°C, 1 minute at 60°C/58°C/54°C, and 1 minute at 68°C; 32/25/25 cycles, respectively).

The kidney samples were studied by staining with hematoxylin and eosin and Masson's stain, and examined by light microscopy. CTGF expression as evaluated by immunohistochemistry. Paraffin-embedded renal tissue sections (4 μm) were deparaffinized and rehydrated, and endogenous peroxidase was blocked by incubating in 3% H_2O_2/methanol (1:1) at 25°C for 30 minutes. The slides were subsequently incubated in phosphate-buffered saline (PBS) solution with 6% serum in 4% bovine serum albumin (BSA) for 1 hour at 37°C to reduce nonspecific background staining, and then incubated overnight at 4°C with primary antibody in PBS containing 1% serum and 4% BSA. After being washed with PBS, the sections were incubated with secondary antibody diluted 1:200 in 4% BSA/PBS for 30 minutes and, after washing, they were revealed by standard techniques. Evaluations were performed by two independent observers in a blinded fashion, and the mean score value was calculated for each rat. Negative controls without the primary antibody, or using an unrelated immunoglobulin G, were included to check for nonspecific staining (not shown).

For western blotting, tissue was homogenized in lysis buffer (50 mmol/L Tris-HCl, 150 mmol/L NaCl, 2 mmol/L ethylenediamine tetraacetic acid, 2 mmol/L EGTA, 0.2% Triton X-100, 0.3% NP-40, 0.1 mmol/L phenyl methyl sulphonyl fluoride, and 1 μg/mL pepstatin A) and then separated by 12% sodium dodecylsulfate-polyacrylamide gel electrophoresis under reducing conditions. After electrophoresis, samples were transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked in PBS containing 0.1% Tween-20 and 5% dry skimmed milk for 1 hour at 37°C, and then incubated in the same buffer with specific CTGF antibody for 18 hours at 4°C. After washing, detection was made by incubation with peroxidase-conjugated secondary antibody, and developed using an enhanced chemiluminescence kit (Amersham). In all experiments, protein content was determined with the bicinchoninic acid method. In addition, Ponceau Red staining was used to show quality of proteins and the efficacy of protein transfer to the membrane (not shown), and tubulin was used as a loading control.

The autoradiographs were scanned with the GS-800 Calibrated Densitometer (Quantity One; BioRad, Madrid, Spain), which obtained densitometric arbitrary units. Data were normalized against those of the corresponding G3PDH or tubulin data. Results are

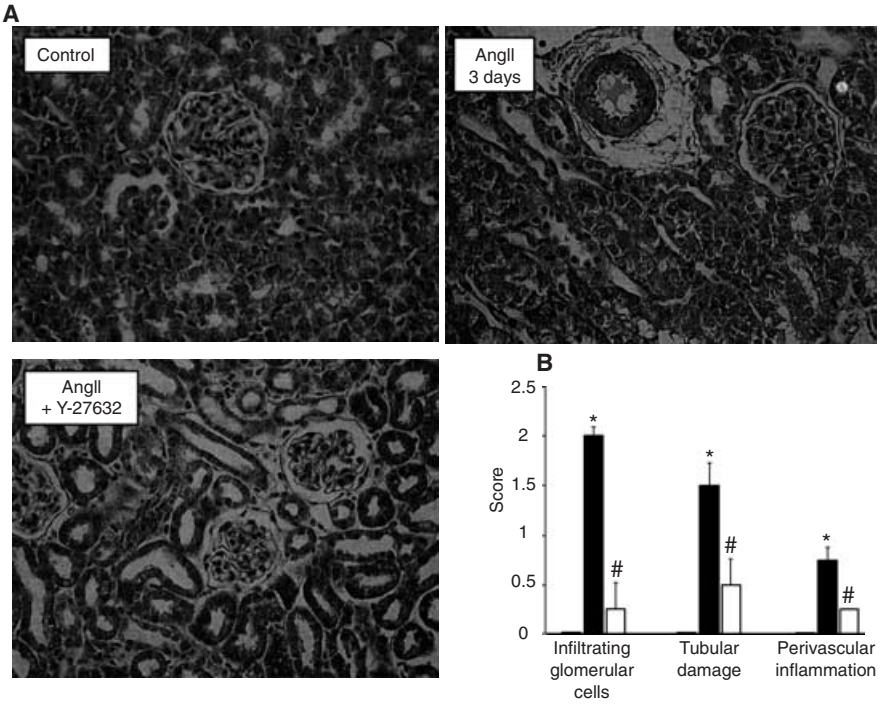

Fig. 1. The Rho-kinase inhibitor Y-27632 ameliorates renal damage caused by systemic infusion of AngII. Animals were treated daily with Y-27632 (30 mg/kg per day), starting 24 hours before AngII infusion (100 ng/kg per minute) and studied after three days. (*A*) The figure shows representative photomicrographs of the kidney from one animal of each group. Magnification 100×. (*B*) The semiquantitative score as mean ± SEM of seven animals of each group. $^*P < 0.05$ vs. control. $\#P < 0.05$ vs. AngII. Control animals showed no renal damage. Black bars represent AngII-infused rats and white bars rats treated with Y-27632.

expressed as *n-fold* increase over control in densitometric arbitrary units, and as mean ± standard error of the mean (SEM) of the experiments undertaken.

Statistical analysis

Significance was established with GraphPAD Instat using Student's *t test* and analysis of variance (GraphPAD Software, San Diego, CA, USA). Differences were considered significant at a value of $P < 0.05$.

RESULTS

Rho-kinase inhibition diminishes renal damage caused by systemic infusion of AngII

Morphologic lesions were examined by light microscopy. After three days of AngII infusion, animals presented inflammatory cells in glomeruli and in tubulointerstitial areas, mild tubular lesions (Fig. 1), but not fibrosis, as previously described [7]. Treatment with the Rho-kinase inhibitor Y-27632 ameliorates renal damage (Fig. 1).

Rho-kinase inhibition diminishes renal overexpression of proinflammatory mediators caused by systemic infusion of AngII

In rats infused with AngII for three days, NF-κB activity was elevated in the kidney. Treatment with Y-27632 partially diminished renal NF-κB DNA binding activity in AngII-infused rats (around 60% inhibition vs. AngII-infusion; electrophoretic mobility shift assay [EMSA], Fig. 2). We evaluated by PCR the gene expression of proinflammatory mediators, such as the cytokine TNF-α and the chemokine MCP-1. AngII-infused rats presented overexpression of these proinflammatory genes that were downregulated by Y-27632 (Fig. 3).

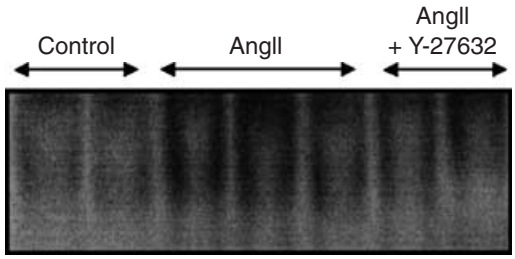

Fig. 2. Rho-kinase inhibition decreased NF-κB activation caused by AngII. Protein extracts were collected and used for gel shift assays with the NF-κB probe. The figure shows a representative EMSA experiment of seven animals of each group. Specificity of the reaction was demonstrated with a 100-fold excess of unlabelled NF-κB oligonucleotide (not shown).

Rho-kinase inhibition diminishes renal CTGF upregulation caused by systemic infusion of AngII

The CTGF gene was upregulated in the kidneys of AngII-infused rats, and CTGF protein production was increased in glomeruli, some tubuli, and renal vessels. Treatment of AngII-infused animals with the Rho-kinase inhibitor Y-27632 diminished CTGF messenger RNA and protein production (immunohistochemistry and Western blot) in all renal structures (Fig. 4).

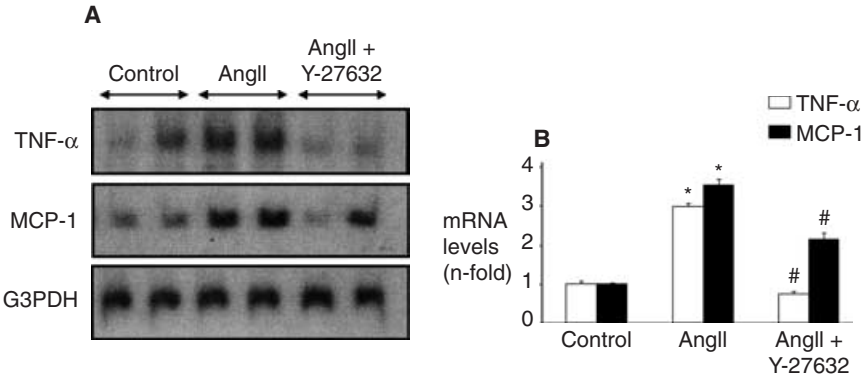

Fig. 3. Rho-kinase inhibition downregulated AngII-induced renal overexpression of proinflammatory mediators. Total cortex RNA was extracted, and gene expression of proinflammatory mediators (TNFα and MCP-1) was analyzed by PCR. Panel *A* shows a representative experiment, and panel *B* shows data as mean ± SEM of seven animals of each group. G3PDH gene expression was used as a control. $^{*}P < 0.05$ vs. control; $\#P < 0.05$ vs. AngII.

DISCUSSION

We observed that treatment with the Rho-kinase inhibitor Y-27632 ameliorates AngII-induced renal damage, diminishing the presence of inflammatory cell infiltration and tubular lesions, as well as the overexpression of proinflammatory and profibrotic mediators. These data suggest the in vivo role of Rho activation in AngII responses. Rho family GTPases are key components of signaling pathways that regulate several cellular functions and processes, such as vasoconstriction, actin reorganization, leukocyte transmigration, and cell growth [16]. RhoA participates in some AngII responses, including vasoconstriction, premyofibril formation, and hypertrophy of cardiomyocytes and vascular smooth muscle cell (VSMC) [11, 12, 15]. Rho-kinase is one of the target proteins of RhoA. In VSMC, Rho-kinase inhibition significantly suppressed AngII-induced hypertrophic response and downregulated AngII-induced genes, including MCP-1 and plasminogen-activator inhibitor-1 [11–15]. In this sense, we have observed that in AngII-infused rats, Rho-kinase inhibition diminished gene overexpression of MCP-1, TNFα, and CTGF. Our data clearly indicate that the Rho/Rho-kinase pathway participates in AngII-mediated renal damage and suggest that treatments that block Rho/Rho-kinase activation could be very useful in pathologic settings associated with elevated local AngII generation. At the doses used, the effects of Y-27632 on AngII-induced renal damage are shown to be responsible for specific inhibition of Rho-kinase, whereas the inhibition of other kinases, such as citron kinase and protein kinase N (PKN), is rarely observed using Y-27632 [26, 27]. Nevertheless, a small in vivo contribution of other Rho effector kinases, besides Rho-associated kinase, cannot be ruled out.

The recruitment of inflammatory cells plays a key role in the onset and progression to irreversible structural renal changes. AngII is a cytokine that participates in the inflammatory response. AngII activates the endothelium and causes the adhesion of monocytes and neutrophils to endothelial, VSMC, and mesangial cells, through the upregulation of adhesion molecules, such as P- and E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 [1]. AngII stimulation of resident renal cells produces cytokines and chemokines, which contributes to the recruitment of infiltrating cells into the kidney, such as MCP-1, the main chemokine of monocytes [1]. The NF-κB pathway plays a key role in the progression of kidney damage. This transcription factor regulates many genes that are vital in the inflammatory process [29]. AngII activates the NF-κB pathway, although depending on the cell type, different AngII receptors are involved [1, 4]. Rho proteins participate in NF-κB activation [30]. We found that Rho-kinase inhibition partially diminished NF-κB activation. We have previously demonstrated that both AT_1 and AT_2 receptors are involved in renal NF-κB activation. In particular, the inhibitory effect observed with the AT_1 antagonists was similar to that found with the Rho-kinase inhibitor, supporting the idea that, through activation of AT_1 and Rho, AngII regulates the NF-κB pathway in the kidney. AngII increases proinflammatory genes under NF-κB control, including adhesion molecules, cytokines, and chemokines. In unilateral ureteral obstruction and nephrectomized spontaneously hypertensive rats, fasudil, another Rho-kinase inhibitor, diminished ED-1-positive cell infiltration [31, 32]. We have demonstrated that Rho-kinase inhibition diminished AngII-induced renal inflammatory response, as shown by lower renal inflammatory cell infiltration, diminution of NF-κB activity, and downregulation of proinflammatory genes, including the cytokine TNF-α and the chemokine MCP-1. These data show that Rho-kinase inhibition regulates the inflammatory response in the kidney.

Several findings suggest a potential role of the Rho/Rho-kinase signaling pathway in the development of fibrotic lesions in various organs, including the kidney. Tubulointerstitial fibrosis is a final common pathway to end-organ damage, and therefore is an important therapeutic target to cure or reverse progressive functional deterioration. In different experimental models of renal damage, such as hypertensive glomerulosclerosis,

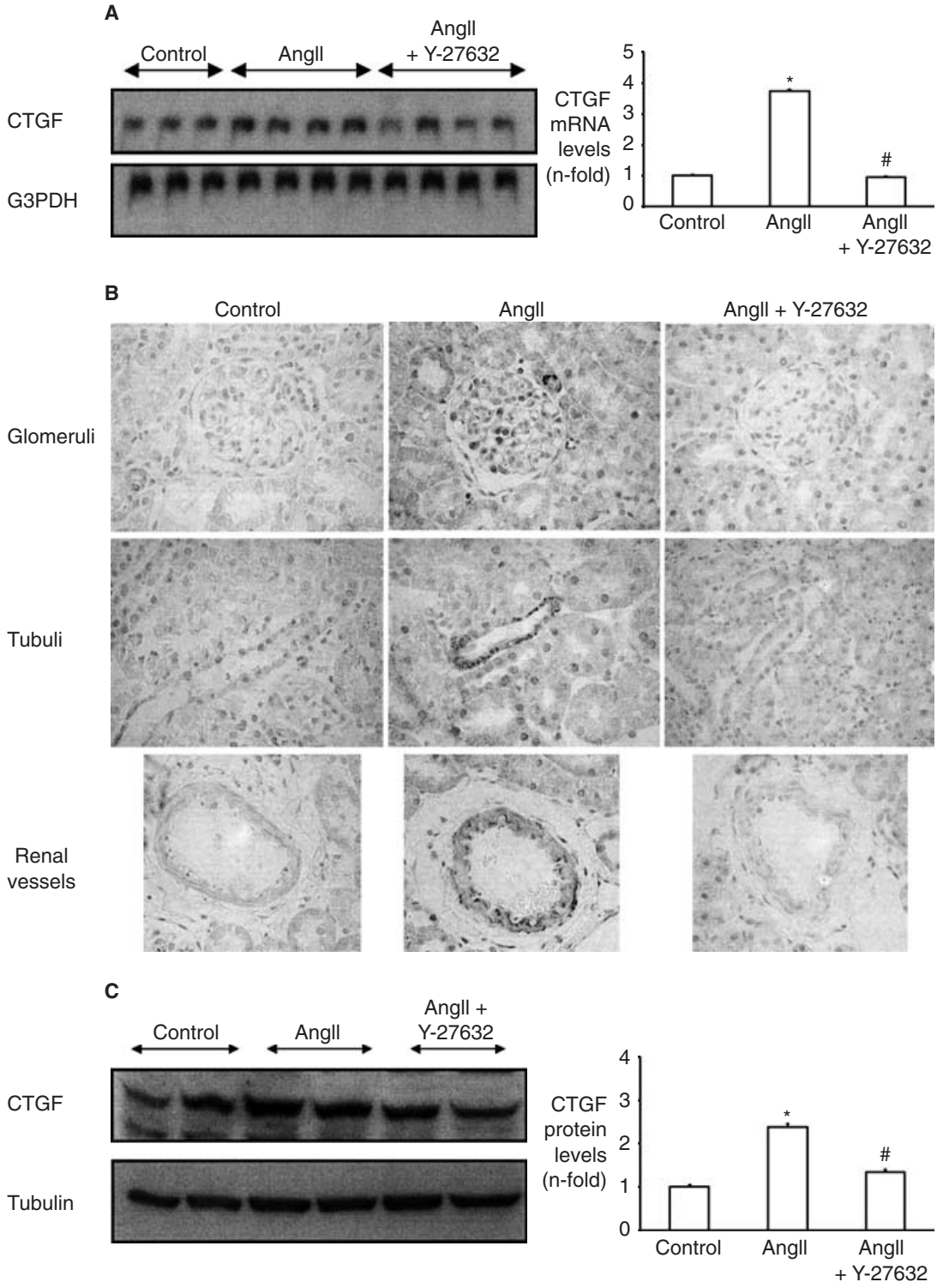

Fig. 4. The Rho-kinase inhibitor Y-27632 diminished CTGF overexpression in the kidney of AngII-infused rats. (*A*) CTGF gene expression determined by reverse-transcriptase polymerase chain reaction. CTGF protein levels were evaluated by immunohistochemistry (*B*) and Western blot (*C*). In A and C, the left panel shows a representative experiment, and the right panel shows the data as mean ± SEM of 7 animals of each group. $^{*}P < 0.05$ vs. control, $\#P < 0.05$ vs. AngII. B shows representative animals of 7 studied in each group. Magnification in *B* 400×.

unilateral ureteral obstruction, nephrectomized spontaneously hypertensive rats, and in rats treated with L-NAME, Rho-kinase inhibition improved glomerular and tubulointerstitial injury scores and fibrosis [24, 31–35]. In nephrectomized spontaneously hypertensive rats, the Rho-kinase inhibitor fasudil ameliorated proliferating cell nuclear antigen-positive cells, through upregulation of p27kip1, a cyclin-dependent kinase inhibitor [31]. In some of these models, Rho-kinase inhibition diminished gene overexpression of smooth muscle α-actin, TGF-β, and extracellular matrix proteins [24, 34]. In rats infused with AngII, we show that Y-27632 diminished tubular damage and overexpression of the profibrotic growth factor CTGF. This growth factor is upregulated in many human renal diseases and contributes to renal fibrosis and tubuloepithelial transdifferentiation [36, 37]. In cultured renal fibroblasts, activation of Rho is involved in TGF-β-induced CTGF overexpression [23] but not in fibroblasts proliferation [24]. Although drugs that block renal AngII production, such as ACE inhibitors and AT_1 antagonists, have demonstrated protective renal effects and can ameliorate fibrosis through CTGF downregulation [10], the data presented here suggest that Rho-kinase inhibitors could be novel targets for renal therapy.

The Rho/Rho-kinase pathway also plays a crucial role in blood pressure regulation in vivo [38]. In different models of hypertension, activation of RhoA has recently been described [39], suggesting that the Rho/Rho-kinase pathway could be a potential mechanism that explains vascular changes in hypertension.

Although different treatments present beneficial effects in renal diseases, novel targets for renal therapy are needed to improve the quality of life of these patients. Many therapeutic treatments currently used, such as ACE inhibitors and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, present pleiotropic effects and target pivotal cell-signaling networks besides the pathways they were initially designed to regulate. In this sense, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors block activation of Rho, showing an intracellular target of their beneficial effects. Our results suggest that renal damage induced by AngII is mediated by activation of the Rho/Rho-kinase pathway, and support the idea that treatments that inhibit this pathway could be an appropriate choice for therapeutic strategies in renal diseases.

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Reprint requests to Marta Ruiz-Ortega, Ph.D., Vascular and Renal Research Laboratory, Fundación Jiménez Díaz, Autonoma University of Madrid, Avda. Reyes Católicos, 2, 28040 Madrid, Spain.
E-mail: mruizo@fjd.es

REFERENCES

1. Ruiz-Ortega M, Lorenzo O, Suzuki Y, *et al*: Proinflammatory actions of Angiotensin II. *Curr Opin Nephrol Hypertens* 10:321–329, 2001
2. Mezzano SA, Ruiz-Ortega M, Egido J: Angiotensin II and renal fibrosis. *Hypertension* 38:635–638, 2001
3. Andersen S, Tarnow L, Rossing P, *et al*: Renoprotective effects of angiotensin II receptor blockade in type 1 diabetic patients with diabetic nephropathy. *Kidney Int* 57:601–606, 2000
4. Ruiz-Ortega M, Ruperez M, Esteban V, Egido J: Molecular mechanisms of angiotensin II-induced vascular injury. *Curr Hypertens Rep* 5:73–79, 2003
5. Johnson RJ, Alpers CE, Yoshimura A, *et al*: Renal injury from angiotensin II-mediated hypertension. *Hypertension* 19:464–474, 1994
6. Wolf G, Ziyadeh FN, Thaiss F, *et al*: Angiotensin II stimulates expression of the chemokine RANTES in rat glomerular endothelial cells. Role of the Angiotensin type 2 receptor. *J Clin Invest* 100:1047–1058, 1997
7. Ruiz-Ortega M, Lorenzo O, Ruperez M, *et al*: Systemic infusion of angiotensin II into normal rats activates nuclear factor k-B and AP-1 in the kidney. Role of AT_1 and AT_2 receptors. *Am J Pathol* 158:1743–1756, 2001
8. Ruiz-Ortega M, Ruperez M, Lorenzo O, *et al*: Angiotensin II regulates the synthesis of proinflammatory cytokines and chemokines in the kidney. *Kidney Int* 82:12–22, 2002
9. Ruperez M, Ruiz-Ortega M, Esteban V, *et al*: Angiotensin II increases connective tissue growth factor in the kidney. *Am J Pathol* 163:1937–1947, 2003
10. Ruperez M, Lorenzo O, Blanco-Colio LM, *et al*: The connective tissue growth factor is a mediator of angiotensin II-induced fibrosis. *Circulation* 108:1499–1509, 2003
11. Yamakawa T, Tanaka S, Numaguchi K, *et al*: Involvement of Rho-kinase in angiotensin II-induced hypertrophy of rat vascular smooth muscle cells. *Hypertension* 35:313–318, 2000
12. Matrougui K, Tanko LB, Loufrani L, *et al*: Involvement of Rho-kinase and the actin filament network in angiotensin II-induced contraction and extracellular signal-regulated kinase activity in intact rat mesenteric resistance arteries. *Arterioscler Thromb Vasc Biol* 21:1288–1293, 2001
13. Takeda K, Ichiki T, Tokunou T, *et al*: Critical role of Rho-kinase and MEK/ERK pathways for angiotensin II-induced plasminogen activator inhibitor type-1 gene expression. *Arterioscler Thromb Vasc Biol* 21:868–873, 2001
14. Funakoshi Y, Ichiki T, Shimokawa H, *et al*: Rho-kinase mediates angiotensin II-induced monocyte chemoattractant protein-1 expression in rat vascular smooth muscle cells. *Hypertension* 38:100–104, 2000
15. Aoki H, Izumo S, Sadoshima J: Angiotensin II activates RhoA in cardiac myocytes: a critical role of RhoA in angiotensin II-induced premyofibril formation. *Circ Res* 82:666–676, 1998
16. Bar-Sagi D, Hall A: Ras and Rho GTPases: A family reunion. *Cell* 103:227–238, 2000
17. Kaibuchi K, Kuroda S, Amano M: Regulation of cytoskeleton and cell adhesion by the Rho family GTPases in mammalian cells. *Annu Rev Biochem* 68:459–486, 1999
18. Amano M, Chihara K, Kimura K, *et al*: Formation of actin stress fiber and focal adhesions enhanced by Rho-kinase. *Science* 275:1308–1311, 1997
19. Fukata Y, Oshiro N, Kinoshita N, *et al*: Phosphorylation of adducin by Rho-kinase plays a crucial role in cell motility. *J Cell Biol* 145:347–361, 1999
20. Kureishi Y, Kobayashi S, Amano M, *et al*: Rho-associated kinase directly induces smooth muscle contraction through myosin light chain phosphorylation. *J Biol Chem* 272:12257–12260, 1997
21. Yasui Y, Amano M, Nagata K, *et al*: Roles of Rho-associated kinase in cytokinesis; mutations in Rho-associated kinase phosphorylation sites impair cytokinetic segregation of glial filaments. *J Cell Biol* 143:1249–1258, 1998
22. Mukai Y, Shimokawa H, Matoba T, *et al*: Involvement of Rho-kinase in hypertensive vascular disease: A novel therapeutic target in hypertension. *FASEB J* 15:1062–1064, 2001

23. Heusinger-Ribeiro J, Eberlein M, Wahab NA, *et al*: Expression of connective tissue growth factor in human renal fibroblasts: Regulatory roles of RhoA and cAMP. *J Am Soc Nephrol* 12:1853–1851, 2001
24. Nagatoya K, Moriyama T, Kawada N, *et al*: Y-27632 prevents tubulointerstitial fibrosis in mouse kidneys with unilateral ureteral obstruction. *Kidney Int* 61:1684–1695, 2002
25. Aznar S, Lacal JC: Searching new targets for anticancer drug design: The families of Ras and Rho GTPases and their effectors. *Prog Nucleic Acid Res Mol Biol* 67:193–234, 2001
26. Fu M, Zhang J, Zhu X, *et al*: Peroxisome proliferator-activated receptor gamma inhibits transforming growth factor beta-induced connective tissue growth factor expression in human aortic smooth muscle cells by interfering with Smad3. *J Biol Chem* 276:45888–45894, 2001
27. Ishizaki T, Uehata M, Tamechika I, *et al*: Pharmacological properties of Y-27632, a specific inhibitor of rho-associated kinases. *Mol Pharmacol* 57: 976–983, 2000
28. Ruiz-Ortega M, Bustos C, Hernández-Presa MA, *et al*: Angiotensin II participates in mononuclear cell recruitment in the kidney through nuclear factor-kappa B activation and monocyte chemoattractant protein-1 gene expression. *J Immunol* 161:430–439, 1998
29. Guijarro C, Egido J: Transcription factor κB (NF-κB) in renal disease. *Kidney Int* 59:415–424, 2001
30. Montaner S, Perona R, Saniger L, Lacal JC: Multiple signalling pathways lead to the activation of the nuclear factor ?B by the Rho family of GTPases. *J Biol Chem* 273:12779–12785, 1998
31. Kanda T, Wakino S, Hayashi K, *et al*: Effect of fasudil on Rho-kinase and nephropathy in subtotally nephrectomized spontaneously hypertensive rats. *Kidney Int* 64:2009–2019, 2003
32. Satoh S, Yamaguchi T, Hitomi A, *et al*: Fasudil attenuates interstitial fibrosis in rat kidneys with unilateral ureteral obstruction. *Eur J Pharmacol* 455:169–174, 2002
33. Teraishi K, Kurata H, Nakajima A, *et al*: Preventive effect of Y-27632, a selective Rho-kinase inhibitor, on ischemia/reperfusion-induced acute renal failure in rats. *Eur J Pharmacol* 505:205–211, 2004
34. Nishikimi T, Akimoto K, Wang X, *et al*: Fasudil, a Rho-kinase inhibitor, attenuates glomerulosclerosis in Dahl salt-sensitive rats. *Hypertens* 22:1787–1796, 2004
35. Kataoka C, Egashira K, Inoue S, *et al*: Important role of Rho-kinase in the pathogenesis of cardiovascular inflammation and remodeling induced by long-term blockade of nitric oxide synthesis in rats. *Hypertension* 39:245–250, 2002
36. Perbal B: CCN proteins: Multifunctional signalling regulators. *Lancet* 363:62–64, 2004
37. Gupta S, Clarkson MR, Duggan J, Brady HR: Connective tissue growth factor: potential role in glomerulosclerosis and tubulointerstitial fibrosis. *Kidney Int* 58:1389–1399, 2000
38. Uehata M, Ishizaki T, Satoh H, *et al*: Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature* 389:990–994, 1997
39. Seko T, Ito M, Kureishi Y, *et al*: Activation of RhoA and inhibition of myosin phosphatase as important components in hypertension in vascular smooth muscle. *Circ Res* 92:411–418, 2003