

Angiotensin II Activates Collagen Type I Gene in the Renal Vasculature of Transgenic Mice During Inhibition of Nitric Oxide Synthesis

Jean-Jacques Boffa, Pierre-Louis Tharaux, Sandrine Placier, Raymond Ardaillou, Jean-Claude Dussaule, Christos Chatziantoniou

▶ **To cite this version:**

Jean-Jacques Boffa, Pierre-Louis Tharaux, Sandrine Placier, Raymond Ardaillou, Jean-Claude Dussaule, et al.. Angiotensin II Activates Collagen Type I Gene in the Renal Vasculature of Transgenic Mice During Inhibition of Nitric Oxide Synthesis. Circulation, 1999, 100 (18), pp.1901-1908. 10.1161/01.cir.100.18.1901 . inserm-03788594

HAL Id: inserm-03788594
https://inserm.hal.science/inserm-03788594

Submitted on 26 Sep 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Angiotensin II Activates Collagen Type I Gene in the Renal Vasculature of Transgenic Mice During Inhibition of Nitric Oxide Synthesis

Evidence for an Endothelin-Mediated Mechanism

Jean-Jacques Boffa, MD; Pierre-Louis Tharaux, MD; Sandrine Placier, BS; Raymond Ardaillou, MD; Jean-Claude Dussaule, MD, PhD; Christos Chatziantoniou, PhD

Background—Hypertension is frequently associated with renal vascular fibrosis. The purpose of this study was to investigate whether angiotensin II (Ang II) is involved in this fibrogenic process.

Methods and Results—Experiments were performed on transgenic mice harboring the luciferase gene under the control of the collagen I-α_2 chain promoter [procolα_2(I)]. Hypertension was induced by chronic inhibition of NO synthesis (N^G-nitro-L-arginine methyl ester, L-NAME). Procolα_2(I) activity started to increase in the renal vasculature after 4 weeks of L-NAME treatment ($P<0.01$) and at 14 weeks reached 3- and 8-fold increases over control in afferent arterioles and glomeruli, respectively ($P<0.001$). Losartan, an AT_1 receptor antagonist, given simultaneously with L-NAME prevented the increase of procolα_2(I) levels and attenuated the development of renal vascular fibrosis without normalizing systolic pressure increase. Because we found previously that endothelin mediated renal vascular fibrosis in the L-NAME model, the interaction between Ang II, endothelin, and procolα_2(I) was investigated in ex vivo and short-term in vivo experiments. In both conditions, the Ang II–induced activation of procolα_2(I) in renal cortex was blocked by an endothelin receptor antagonist.

Conclusions—During chronic inhibition of NO, the collagen I gene becomes activated, leading to the development of renal vascular fibrosis. Ang II is a major player in this fibrogenic process, and its effect on collagen I gene is independent of systemic hemodynamics and is at least partly mediated by the profibrogenic action of endothelin. **(*Circulation*. 1999;100:1901-1908.)**

Key Words: hypertension ■ kidney ■ collagen ■ angiotensin ■ endothelin

Downloaded from http://ahajournals.org by on September 29, 2022

One of the most common complications associated with hypertension is the development of renal sclerotic injury.[1] This pathophysiological process is characterized by structural changes in the renal vasculature, mainly due to collagen type I accumulation in renal resistance vessels, glomeruli, and interstitium.[2]

Several studies support a central role for the renin-angiotensin system in the development of renal fibrosis.[3,4] Although it is well established that blockade of ACE slows progression of renal fibrosis, it remains unclear whether this protective effect is solely due to reduction of renal vascular resistance or whether other, pressure-independent, mechanism(s) are operating.[5] Recent studies indicated that endothelial vasoactive agents such as NO and endothelin could be involved in this pathophysiological process. In this regard, chronic inhibition of NO synthesis was accompanied by renal vascular fibrosis,[6,7] whereas endothelin antagonism was accompanied by reversal of vascular hypertrophy and fibrosis in several forms of experimental hypertension.[8–10] In previous studies, we investigated the role of NO and endothelin in the mechanisms of renal vascular fibrosis.[11] We have observed that in the hypertension induced by NO deficiency, the collagen I gene is highly activated in the renal vasculature and that endothelin receptor antagonism prevented this activation.

In the present studies, we evaluated whether angiotensin II (Ang II) plays a role in the mechanism(s) controlling the development of renal vascular fibrosis during hypertension and if so, how its effect could be integrated with the profibrogenic effect of endothelin found in our previous studies. To this end, hypertension was induced by inhibiting NO synthesis in transgenic mice harboring the luciferase gene under the control of the collagen I-α_2 chain promoter [procolα_2(I)], and the activation of collagen I gene was estimated in afferent arterioles, glomeruli, renal cortex, heart, and aorta. The role of Ang II in the fibrogenic process was

Received May 4, 1999; revision received June 16, 1999; accepted June 17, 1999.

From INSERM U.489, Hôpital Tenon, and AP-HP, Laboratoire de Physiologie, Faculté de Médecine St Antoine (J.-C.D.), Paris, France.

Reprint requests to Christos Chatziantoniou, PhD, INSERM U.489, Hôpital Tenon, Paris 75020, France. E-mail christos.chatziantoniou@tnn.ap-hop-paris.fr

© 1999 American Heart Association, Inc.

Circulation **is available at http://www.circulationaha.org**

assessed by use of pharmacological blockade of Ang II type 1 receptors (AT_1) in vivo. In addition, the interaction between Ang II and endothelin on collagen I gene activation was tested by use of endothelin receptor antagonism in acute ex vivo and in vivo experiments.

Methods

Animal Treatment

Male transgenic mice weighing 25 to 35 g (3 to 6 months old) were maintained on a normal-salt diet. This transgenic line, named pGB 19.5/13.5, was generated in the laboratory of B. de Crombrugghe (University of Texas, Houston).[12] These animals harbor the sequences −19.5 to −13.5 kb and −350 to +54 bp of the promoter of the α_2-chain of mouse collagen type I gene linked to the firefly luciferase and the *Escherichia coli* β-galactosidase reporter genes.

To inhibit NO synthesis, mice were treated with N^G-nitro-L-arginine methyl ester (L-NAME), an NO synthase inhibitor (20 mg · $kg^{-1} \cdot d^{-1}$). In a separate group of control or L-NAME–treated mice, a low dose of losartan (AT_1 antagonist) was administered orally (10 mg · $kg^{-1} \cdot d^{-1}$). In preliminary experiments, we found that this dose was inefficient in preventing hypertension in mice.

Isolation of Afferent Arterioles and Glomeruli

Afferent arterioles and glomeruli were isolated according to a protocol similar to that previously described.[11] Kidneys from 4 mice were used to isolate afferent arterioles and glomeruli in each experiment.

Assays for the Expression of Luciferase

Luciferase activity was measured in afferent arterioles, glomeruli, renal cortical slices, abdominal aorta, heart, tail, and skin with a commercial kit (Boehringer Mannheim) and a Lumat LB 9507 luminometer (EG & Berthold) as previously described.[11] Results are expressed as luciferase light units per μg protein (LU/μg).

Measurement of Blood Pressure

Systolic blood pressure was measured by the tail-cuff method adapted to the mouse as previously described[11] with a piezoelectric sensor (Sensonor 840-01) connected to a MacLab/4s 16-bit analog-to-digital converter (ADInstruments) and to a Power PC Macintosh 4400/200 computer. Pressure recording was analyzed with MacLab software.

Renal Histology

Kidneys from ≥3 mice from each group were fixed in Dubosq solution. Three cortical slices of each kidney were embedded in paraffin, and sections 3 μm thick were stained with Masson's trichrome solution for specific staining of extracellular matrix proteins.

Morphological Evaluation

Sections of kidneys were examined on a blinded basis for the level of glomerular and microvascular injury using the 0 to 4+ injury scale. Injury scale 0 means normal extracellular matrix deposition in glomeruli, whereas 1+, 2+, 3+, and 4+ correspond to 1% to 25%, 26% to 50%, 51% to 75%, and 76% to 100% of glomeruli expressing increased extracellular matrix deposition per section, respectively. Thirty to 40 samples (containing ≥20 glomeruli per sample) were studied in each group.

Measurement of Endothelin Excretion

Urine samples from the control, the 14-week L-NAME, and the 14-week L-NAME+losartan groups were collected from the bladder. Immunoreactive endothelin-1 was measured by ELISA (Biomedica).[13] Values were expressed as pg endothelin/μmol creatinine.

Ex Vivo Addition of Ang II and of Endothelin

Renal cortical slices were isolated from control animals and incubated in RPMI medium for 2 hours at room temperature. Ang II or endothelin (10^{-8} mol/L) was added in the incubation medium either alone or in combination with losartan or bosentan (10^{-7} mol/L). Luciferase activity was measured as described above.

Acute Administration of Ang II In Vivo

In a separate group of animals (n=16), Ang II was injected intraperitoneally (1 nmol IP). Measurements of systolic blood pressure followed by isolation of renal cortical slices were performed at 4, 24, and 48 hours after injections. In a subgroup of these studies, losartan (10 nmol IP) or bosentan (10 nmol IP) was injected either alone or mixed with Ang II, and luciferase activity was measured as described above.

Statistical Methods

Statistical analyses were performed by use of ANOVA followed by Fisher's protected least significant difference test (Statview). Results with $P<0.05$ were considered statistically significant. All values are mean±SEM.

Results

Transgenic Model

The choice of these mice was based on previous studies showing that the expression pattern of luciferase in embryos and adult animals correlates closely with cell and tissue distribution of collagen I.[11,12] For instance, tissues rich in collagen I (tail, skin) showed very high levels of luciferase activity; tissues poor in collagen I (renal cortex, afferent arterioles) showed little activity; and where collagen I is almost absent (glomeruli), there was negligible luciferase activity (Reference 11, Table 1).

Effects of L-NAME Treatment on Procolα_2(I) Gene Activation

Systolic blood pressure started rising after 6 weeks of L-NAME treatment and reached a plateau at ≈160 mm Hg after 10 weeks of treatment ($P<0.01$, Table 1). Early in the development of hypertension (6 weeks), renal cortical structure did not exhibit abnormal extracellular matrix accumulation (Figure 1A). On the contrary, renal vascular and glomerular fibrosis was evident at 14 weeks (Figure 1B). Semiquantitative evaluation of extracellular matrix formation confirmed renal injury in L-NAME–treated mice (Table 2). Glomeruli with scores from 2+ to 4+ were rare in controls, whereas they represented 25% of the glomeruli in animals after 14 weeks of treatment.

Inhibition of NO synthesis increased luciferase activity in the renal vasculature before the onset of blood pressure increase: isolated glomeruli displayed a 2-fold increase of luciferase activity after 4 weeks of L-NAME treatment ($P<0.05$, Table 1). The L-NAME–induced activation of procolα_2(I) was further increased with time and reached an 8-fold increase versus control after 14 weeks of treatment (Table 1). Similarly, luciferase activity in afferent arterioles started increasing after 4 weeks of L-NAME treatment and reached a 3-fold increase at 14 weeks ($P<0.05$, Table 1). This early activation of collagen I gene was specific to renal vessels. Luciferase activity in aorta and heart of L-NAME–treated mice was unchanged in the initial phase of hypertension (up to 10 weeks), and it increased thereafter (Table 1).

Downloaded from http://ahajournals.org by on September 29, 2022

TABLE 1. Time Course of Systolic Blood Pressure and Luciferase Activity in Vascular Tissues Isolated From Transgenic Mice During NO Deficiency

	Time of Treatment, wk						
	0	4	6	8	10	12	14
Systolic pressure, mm Hg							
Control	116±2	118±2	121±3	119±3	118±4	122±4	119±3
L-NAME	118±2	119±2	130±2*	142±3*	155±5*	160±5*	162±5*
Luciferase activity, LU/μg							
Afferent arterioles	179±4	259±10*	341±22*	437±20*	485±45*	540±55*	605±54*
Glomeruli	20±2	38±3*	55±3*	102±11*	122±5*	127±15*	150±13*
Renal cortex	167±5	175±7	216±12*	289±15*	327±20*	394±28*	450±26*
Heart	136±10	121±15	125±14	140±14	218±15*	332±18*	326±18*
Aorta	1647±107	1677±151	1564±105	1896±178	3300±145*	4032±327*	4322±522*

Values are mean±SEM; n=16 animals per group.
*$P<0.05$ vs control.

L-NAME treatment did not change luciferase activity in 2 control (nonvascular, rich in collagen I) tissues, tail and skin (data not shown).

Effects of AT-1 Antagonism on Procolα_2(I) Gene Activation

AT_1 antagonism by losartan did not attenuate the increase of systolic blood pressure in L-NAME–treated mice at 8 weeks; it had a moderate antihypertensive effect thereafter (−10%), but these mice remained hypertensive compared with age-matched controls ($P<0.05$, Figure 2 and Table 1).

Losartan administration did not modify luciferase activity in control animals (Figures 3 and 4). In contrast, losartan prevented the L-NAME–induced activation of procolα_2(I) in afferent arterioles, glomeruli, and renal cortex at 8 and 14 weeks (Figure 3). The inhibitory effect of losartan on L-NAME–induced activation of procolα_2(I) was also observed in aorta and heart in the late phase of hypertension (14 weeks, Figure 4).

Antagonism of AT_1 receptors markedly protected kidneys from the L-NAME–induced fibrosis, as evidenced by the attenuated levels of extracellular matrix staining in the L-NAME+losartan group (Figure 5, Table 2).

In agreement with our previous studies,[11,13] urinary excretion of endothelin was significantly elevated after 14 weeks of L-NAME treatment (25.4±3.9 versus 12.3±1.7 fmol/μmol creatinine, $P<0.01$, for L-NAME versus control mice, respectively). Interestingly, losartan blunted this increase of endothelin excretion (16.8±2.0 fmol/μmol creatinine for L-NAME+losartan–treated mice).

Ang II and Endothelin-Induced Activation of the Procolα_2(I) Gene Ex Vivo

To examine whether Ang II can induce collagen type I gene activation independently of systemic hemodynamics, renal cortical slices were incubated in the presence of Ang II in vitro. As shown in Figure 6 (top), Ang II produced a 2- to

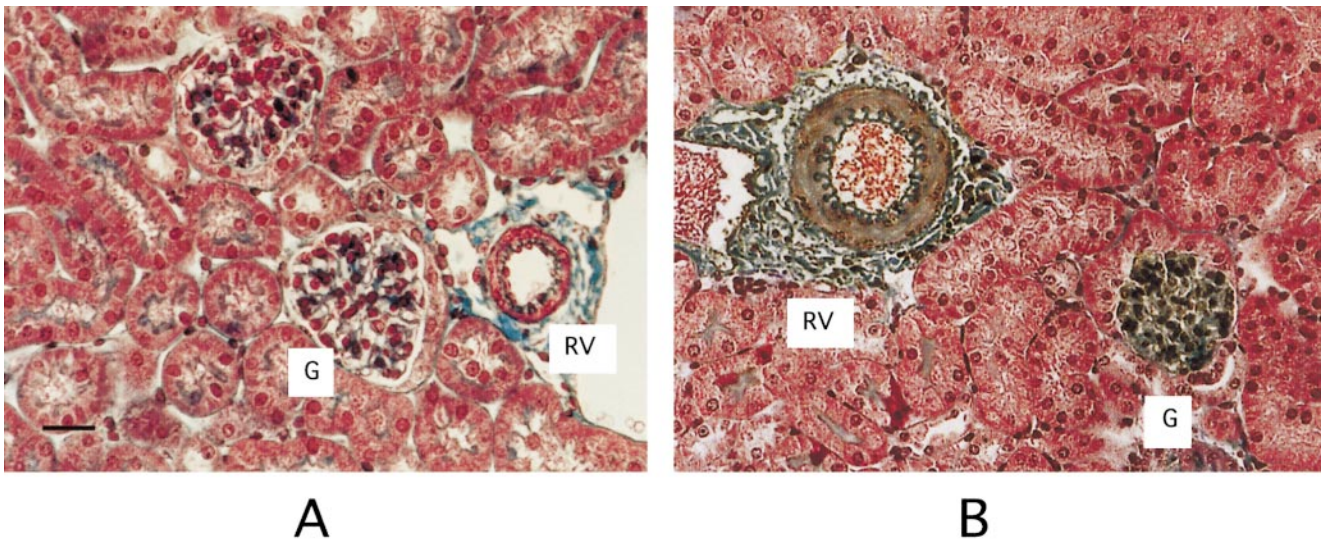


Figure 1. Extracellular matrix staining by Masson's trichrome solution in mouse treated with L-NAME for 6 (A) and 14 (B) weeks. Note normal aspect of glomerulus (G) and renal microvessel (RV) at 6 weeks and intense green staining in these tissues at 14 weeks. Bar=10 μm.

Downloaded from http://ahajournals.org by on September 29, 2022

TABLE 2. Extracellular Matrix Deposition in Glomeruli Isolated From Controls and Mice Treated With L-NAME or L-NAME+losartan for 14 Weeks

	Extracellular Matrix Score				
	0	1+	2+	3+	4+
Control	95±2	3±1	2±1	...	...
L-NAME	48±8*	27±4*	14±3*	7±1*	4±1*
L-NAME+losartan	85±3*†	10±2*†	5±1*†	...	...

Numbers are % of total glomerular sections.
*$P<0.05$ vs control; †$P<0.05$ L-NAME+losartan vs L-NAME–treated group.

3-fold increase in luciferase activity in renal cortical slices ($P<0.01$), and this increase was completely prevented by losartan.

To test whether the stimulatory effect of Ang II on procolα_2(I) activation was mediated by endothelin, an endothelin receptor antagonist, bosentan, was used instead of losartan. Bosentan almost completely blocked the Ang II–induced increase of luciferase activity (Figure 6, bottom).

Addition of endothelin to renal cortical slices produced a 2-fold increase of collagen I gene, and this effect of endothelin was inhibited by bosentan (Figure 6, bottom). However, losartan had no effect on the endothelin-induced procolα_2(I) activation (Figure 6, top).

Ang II–Induced Activation of the Procolα_2(I) Gene In Vivo

This set of experiments was performed to verify that Ang II can acutely induce collagen type I gene activation in the renal tissue in vivo and to test the role of endothelin as mediator of this action. To this end, Ang II alone or mixed with losartan or bosentan was injected intraperitoneally into control animals.

Exogenous Ang II slightly increased luciferase activity in renal cortical slices 4 hours after the injections. Luciferase activity was further increased 24 or 48 hours after Ang II administration (147±10 versus 185±22, 387±42, and 410±35 LU/μg for control, 4, 24, and 48 hours, respectively), whereas systolic blood pressure did not change (115±3, 119±3, 114±5, and 112±4 mm Hg for similar periods of time, respectively). The effect of Ang II on luciferase activity was blocked by losartan (Figure 7, top). In addition, the Ang II–induced activation of collagen I gene was significantly blunted by bosentan (Figure 7, bottom), further suggesting that endothelin mediates the Ang II–induced collagen I gene activation.

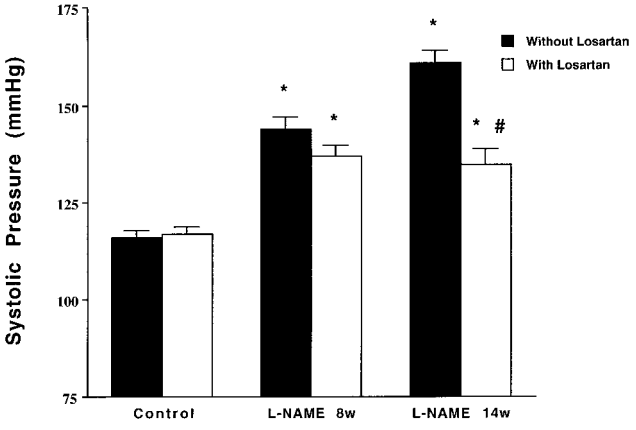


Figure 2. Systolic blood pressure measured in controls and mice treated with losartan, L-NAME, and L-NAME+losartan for 8 or 14 weeks. Values are mean±SEM of 16 mice per group. *$P<0.05$ vs control; #$P<0.05$ L-NAME vs L-NAME+losartan–treated group.

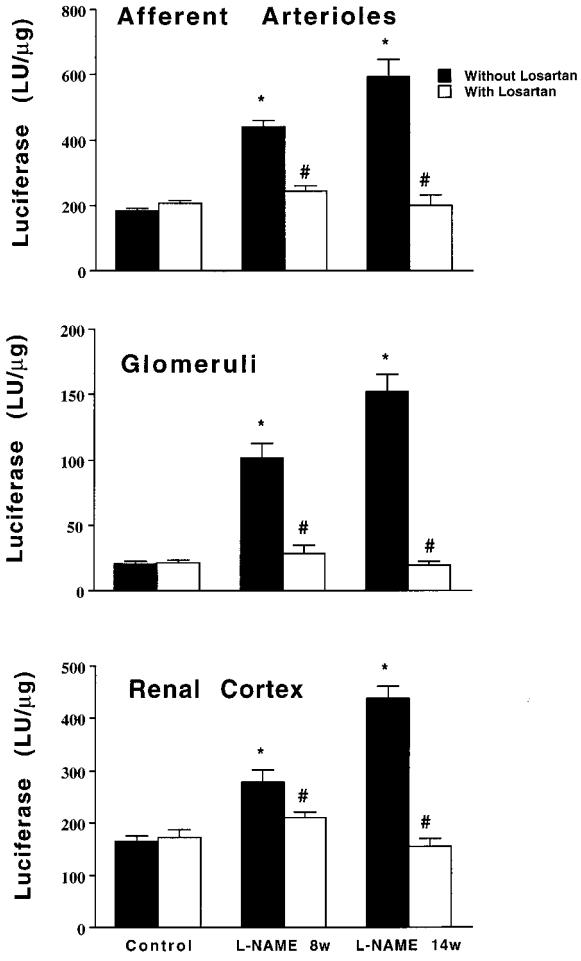


Figure 3. Luciferase activity from afferent arterioles (top), glomeruli (middle), and renal cortical slices (bottom) isolated from controls and mice treated with losartan, L-NAME, and L-NAME+losartan for 8 or 14 weeks. Values are mean±SEM of 16 experiments. *$P<0.05$ vs control; #$P<0.05$ L-NAME vs L-NAME+losartan–treated group.

Discussion

In the present study, a strain of transgenic mice harboring the luciferase reporter gene under the control of collagen I promoter permitted us to investigate early mechanisms in the development of renal fibrosis and to reveal a complex interaction between NO, Ang II, and endothelin that controls the activation of collagen type I gene in the renal vasculature. Specifically, we found that NO blockade was accompanied by an early increase of procolα_2(I) and that AT_1 antagonism abolished this activation of procolα_2(I). The capability of Ang II to activate collagen I gene in the renal tissue was corrob-

Downloaded from http://ahajournals.org by on September 29, 2022

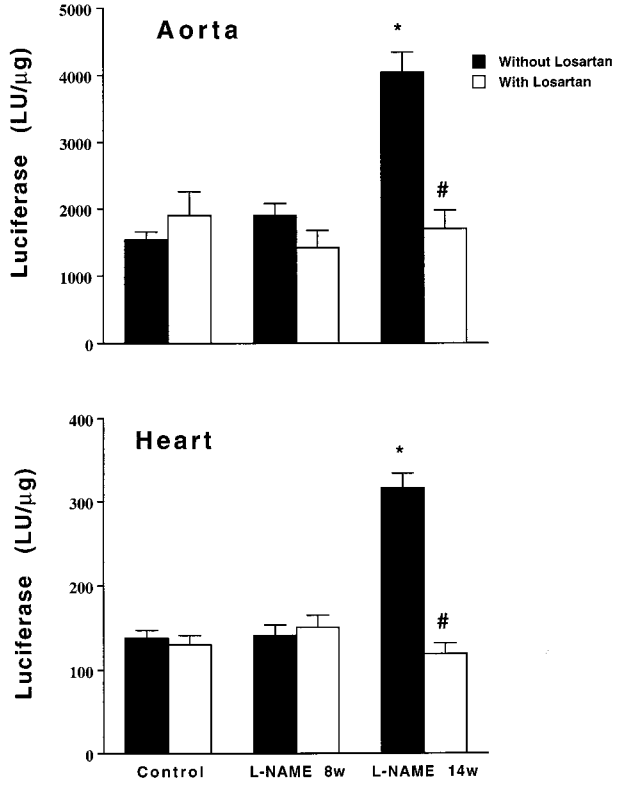


Figure 4. Luciferase activity from aorta (top) and heart (bottom) isolated from controls and mice treated with losartan, L-NAME, and L-NAME+losartan for 8 or 14 weeks. Values are mean±SEM of 16 experiments. *$P<0.05$ vs control; #$P<0.05$ L-NAME vs L-NAME+losartan–treated group.

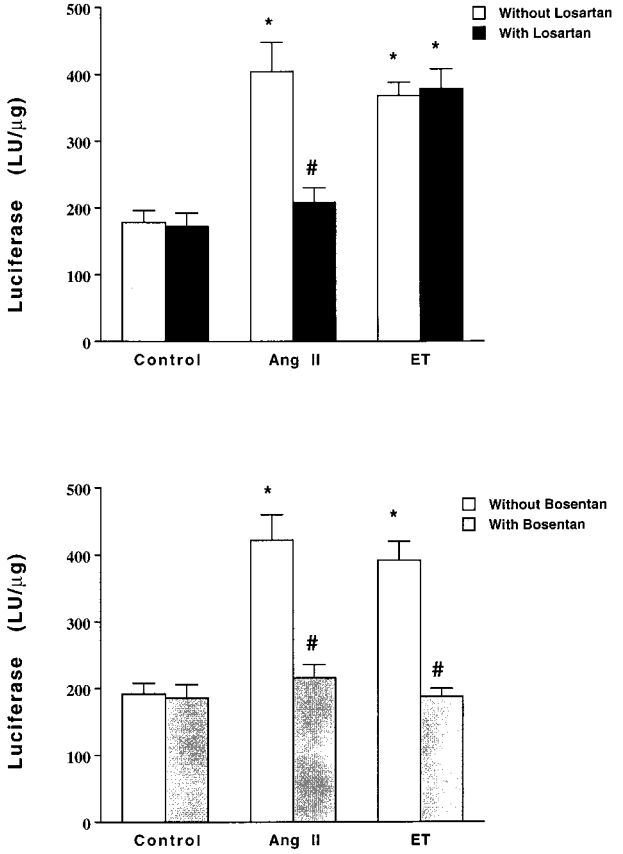


Figure 6. Luciferase activity in renal cortical slices under control conditions and in presence of Ang II or endothelin with or without losartan (top) or bosentan (bottom). Values are mean±SEM of 10 experiments. *$P<0.05$ vs control; #$P<0.05$ vs Ang II or endothelin.

orated by ex vivo and acute in vivo experiments. Interestingly, the Ang II–induced activation of procolα_2(I) was blocked by endothelin receptor antagonism, suggesting that the fibrogenic effect of Ang II is mediated by endothelin.

The development of hypertension induced by chronic inhibition of NO synthesis is accompanied by structural damage of the renal and cardiac vasculature, including vascular wall thickening, macrophage invasion, and myocardial, perivascular, glomerular, and interstitial fibrosis.[10,14,15] In our case, renal histological lesions were not detectable up to 8 weeks. They became evident after 10 weeks, but

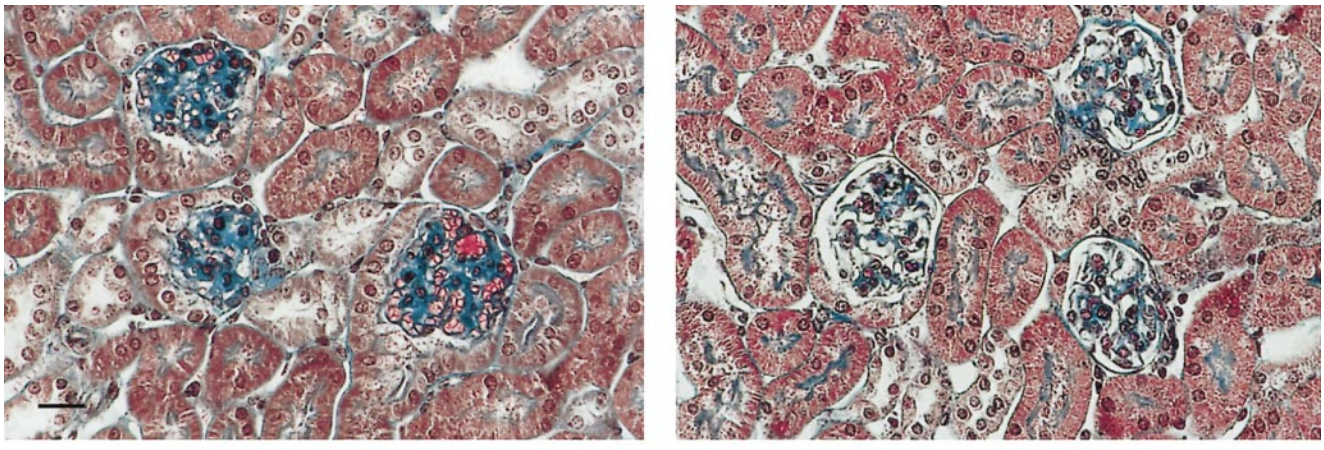


Figure 5. Extracellular matrix deposition by Masson's trichrome solution in renal cortical slices from mice treated for 14 weeks with L-NAME (A) and L-NAME+losartan (B). Note that losartan blunted intense green staining of extracellular matrix. Bar=10 μm.

Downloaded from http://ahajournals.org by on September 29, 2022

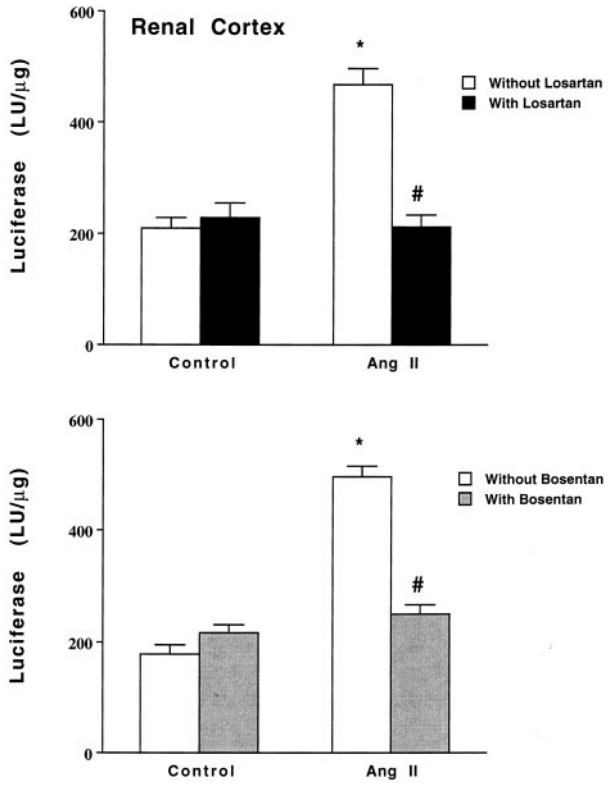


Figure 7. Effect of AT_1 and endothelin antagonism on Ang II–induced collagen I gene activation in renal cortical slices in vivo. Values are mean±SEM of 12 experiments. *$P<0.05$ vs control; #$P<0.05$ vs Ang II–injected group.

remained moderate compared with what has been observed in the rat, probably as a result of species differences.

Several recent reports imply that a local, de novo generation of Ang II at sites of repair plays a central role in the fibrogenic mechanisms.[16] Ang II stimulated collagen protein synthesis in cultured cardiac fibroblasts in vitro and increased collagen I mRNA expression in rat hearts in vivo,[17–19] whereas inhibition of NO produced an early increase of cardiac ACE activity and cardiac Ang II receptor expression and content.[15,20] Use of ACE inhibitors or AT_1 antagonists markedly reduced the development of L-NAME–induced cardiac and renal fibrosis and improved survival.[20–22] In agreement with these studies, AT_1 antagonism completely prevented collagen I gene activation and attenuated the degree of fibrosis in the kidneys of our transgenic mice (Figures 3 through 5).

In several of the above-mentioned studies, the fibrogenic action of Ang II was independent of hemodynamic loading. Thus, collagen type I gene expression was also increased in the right ventricle, a compartment exposed to relatively low systolic pressure,[19] whereas ACE inhibition, but not hydralazine, prevented vascular and myocardial remodeling in L-NAME–treated rats, despite the similarity of systolic blood pressure levels in these 2 groups.[20] Our data provide 3 elements supporting the hypothesis that Ang II induces renal vascular fibrosis through a systemic pressure-independent operating mechanism. First, the activation of collagen I gene was observed earlier (4 weeks) than the onset of high blood pressure (6 weeks). Second, AT_1 antagonism normalized procolα_2(I) expression (at 8 or 14 weeks) without normalizing the L-NAME–induced increase in systolic pressure (Figures 2 and 3). Finally, the ex vivo (in which hydrostatic pressure is not a factor, Figure 6) and the acute in vivo experiments (in which systolic pressure was not elevated when luciferase activity was increased, see Ang II–Induced Activation of the Procolα_2(I) Gene In Vivo and Figure 7) indicate that Ang II is capable of inducing collagen I gene independently of systemic hemodynamics.

Another vasoactive agent that appears to be activated during NO synthesis inhibition is endothelin. We have previously observed that endothelin and collagen I mRNA expression and synthesis were concomitantly increased in renal resistance vessels in rats treated with L-NAME.[13] Antagonism of endothelin receptors by bosentan abolished the exaggerated expression and synthesis of collagen I and markedly blunted the induction of fibrosis in the renal vasculature. Interestingly, bosentan prevented the increase of procolα_2(I) activity without attenuating the L-NAME–induced increase of systolic pressure,[11] suggesting an endothelin-mediated activation of collagen I gene independent of systemic hemodynamics.

To integrate our previous data with the new data, we propose that the stimulatory effect of Ang II on collagen I gene activation is mediated by the action of endothelin. An initial argument for this hypothesis is the observation that AT_1 antagonism attenuated the increased urinary levels of endothelin at 14 weeks. The acute ex vivo and in vivo experiments offer further support for this hypothesis. In both cases, the stimulatory effect of Ang II on procolα_2(I) was completely blocked by bosentan (Figures 6 and 7, bottom), whereas losartan had no effect on the endothelin-induced activation of collagen I gene (Figure 6). In addition, the ex vivo experiments indicate that the interaction between Ang II, endothelin, and procolα_2(I) activation can occur independently of systemic hemodynamics, whereas the experiments with the exogenous intraperitoneal administration of Ang II demonstrate the feasibility of this interaction in vivo.

Several recent lines of evidence are in favor of an Ang II–induced activation of endothelin in vascular tissues. In the model of Ang II–induced hypertension, endothelin content or immunostaining was increased in aorta, femoral artery, and kidney; antagonism of Ang II receptors with losartan normalized endothelin levels.[23,24] Treatment of hypertensive animals with endothelin receptor antagonists attenuated the degree of renal lesions without affecting systemic blood pressure.[9,25] Similar observations were made in other models of vascular injury in which the renin-angiotensin system is a major pathophysiological factor such as congestive heart failure, uninephrectomized spontaneously hypertensive rats, or chronic renal failure (⅚ nephrectomy).[26–28] In these studies, endothelin expression or peptide levels were increased (in particular in renal microvessels and glomeruli); treatment with an ACE inhibitor or AT_1 antagonist blunted the development of the lesion and was always accompanied by inhibition of endothelin activation. It has also been reported recently that therapy with losartan in hypertensive patients was accompanied by a significant decrease of endothelin

Downloaded from http://ahajournals.org by on September 29, 2022

plasma levels.[29] All these studies clearly indicate that in some experimental models, long-term effects of Ang II on vascular structure are mediated by endothelin. It is possible that this interaction implies transforming growth factor (TGF)-β, because it is considered one of the most potent signals for the induction of collagen I gene activation and renal fibrosis.[30] In this regard, expression of TGF-β and of extracellular matrix proteins was increased in cardiac fibroblasts of rats treated with L-NAME, and these increases were completely prevented by AT_1 antagonism.[15,31]

The observation that endothelin is an important mediator of the fibrogenic action of Ang II can have important implications in the treatment of nephroangiosclerosis and glomerulosclerosis in human essential hypertension. AT_1 receptor antagonists and ACE inhibitors reduce proteinuria and protect renal function in renal diseases. However, their limited efficiency enhances the idea of using novel drugs, such as endothelin receptor antagonists, against the development of renal sclerotic injury in hypertension. Thus, it would be interesting to test whether combined therapy with endothelin receptor antagonists and ACE inhibitors or AT_1 antagonists can improve renal function during hypertension, as appears to be the case with pulmonary and heart function in chronic heart failure.[32]

In conclusion, we used a new model of transgenic mouse to investigate the development of renal vascular and glomerular fibrosis. NO participates in the mechanisms controlling collagen I gene expression under normal conditions in renal resistance vessels, because chronic inhibition of NO synthesis induced the activation of collagen I gene. This local activation is probably independent of systemic hemodynamics. Ang II appears to be a major player, because AT_1 receptor antagonism prevented the activation of collagen I gene. Interestingly, this fibrogenic action of Ang II is not direct and seems to implicate the mediation of endothelin. These data indicate the importance of the interactions between the renin-angiotensin system and the endothelial vasodilators and vasoconstrictors in the pathophysiological mechanisms controlling extracellular matrix synthesis.

Acknowledgments

This work was financially supported by the Institut National de la Santé et de la Recherche Médicale and the Faculté de Médecine St Antoine. Drs Boffa and Tharaux were research fellows of the Fondation pour la Recherche Médicale and of the Groupe de Reflexion sur le Système Cardiovasculaire, respectively. The authors thank Drs Bou-Gharios, Rossert, and de Crombrugghe (Department of Molecular Genetics, University of Texas, Houston), MSD-France, and Hoffman–La Roche for providing the transgenic mice, losartan, and bosentan, respectively.

References

1. Weistuch JM, Dworkin LD. Does essential hypertension cause end-stage renal disease? *Kidney Int*. 1992;41:S33–S37.
2. Yoshioka K, Tohda M, Takemura T, Akano N, Matsubara K, Ooshima A, Maki S. Distribution of the type I collagen in human kidney diseases in comparison with type III collagen. *J Pathol*. 1990;162: 141–148.
3. Anderson S, Meyer TW, Renke HG, Brenner BM. Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. *J Clin Invest*. 1985;76:612–619.
4. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin converting enzyme inhibition on diabetic nephropathy. *N Engl J Med*. 1993;329:1456–1462.
5. Border WA, Noble NA. Interactions of transforming growth factor-β and angiotensin II in renal fibrosis. *Hypertension*. 1998;31(pt 2):181–188.
6. Xu Y, Arnal JF, Hinglais N, Appay MD, Laboulandine I, Bariety J, Michel JB. Renal hypertensive angiopathy: comparison between chronic NO suppression and DOCA-salt intoxication. *Am J Hypertens*. 1995;8:167–176.
7. Baylis C, Mitruka B, Deng A. Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J Clin Invest*. 1992;90:278–281.
8. Li JS, Larivière R, Schiffrin EL. Effect of a nonselective endothelin antagonist on vascular remodeling in deoxycorticosterone acetate–salt hypertensive rats: evidence for a role of endothelin in vascular hypertrophy? *Hypertension*. 1994;24:183–188.
9. Moreau P, d'Uscio LV, Shaw S, Takase H, Barton M, Lüscher TF. Angiotensin II increases tissue endothelin and induces vascular hypertrophy: reversal by ET_A-receptor antagonist. *Circulation*. 1997;96: 1593–1597.
10. Bouriquet N, Dupont M, Herizi A, Mimran A, Casellas D. Preglomerular sudanophilia in L-NAME hypertensive rats: involvement of endothelin. *Hypertension*. 1996;27:382–391.
11. Chatziantoniou C, Boffa JJ, Ardaillou R, Dussaule JC. Nitric oxide inhibition induces early activation of type I collagen gene in renal resistance vessels and glomeruli in transgenic mice: role of endothelin. *J Clin Invest*. 1998;101:2780–2789.
12. Bou-Gharios G, Garrett LA, Rossert J, Niederreither K, Eberspaecher H, Smith C, Black C, de Crombrugghe B. A potent far-upstream enhancer in the mouse proα_2(I) collagen gene regulates expression of reporter genes in transgenic mice. *J Cell Biol*. 1996;134:1333–1344.
13. Tharaux PL, Chatziantoniou C, Casellas D, Fouassier L, Ardaillou R, Dussaule JC. Vascular endothelin-1 gene expression, synthesis, and effect on renal type I collagen synthesis and nephroangiosclerosis during nitric oxide synthase inhibition in rats. *Circulation*. 1999;99: 2185–2191.
14. Namaguchi K, Egashira K, Takemoto M, Kadokami T, Shimokawa H, Sueishi K, Takeshita A. Chronic inhibition of nitric oxide synthesis causes coronary microvascular remodeling in rats. *Hypertension*. 1995;26:957–962.
15. Katoh M, Egashira K, Usui M, Ichiki T, Tomita H, Shimokawa H, Rakugi H, Takeshita A. Cardiac angiotensin II receptors are upregulated by long-term inhibition of nitric oxide synthesis in rats. *Circ Res*. 1998;83:743–751.
16. Weber K. Extracellular matrix remodeling in heart failure: a role for de novo angiotensin II generation. *Circulation*. 1997;96:4065–4082.
17. Crawford DC, Chobanian AV, Brecher P. Angiotensin II induces fibronectin expression associated with cardiac fibrosis in the rat. *Circ Res*. 1994;74:727–739.
18. Brilla CG, Zhou G, Matsubara L, Weber KT. Collagen metabolism in cultured adult rat cardiac fibroblasts: response to angiotensin II and aldosterone. *J Mol Cell Cardiol*. 1994;26:809–820.
19. Ju H, Dixon IMC. Effect of angiotensin II on myocardial collagen gene expression. *Mol Cell Biochem*. 1996;163/164:231–237.
20. Takemoto M, Egashira K, Usui M, Numaguchi K, Tomita H, Tsutsui H, Shimokawa H, Sueishi K, Takeshita A. Important role of tissue angiotensin converting enzyme activity in the pathogenesis of coronary vascular and myocardial structural changes induced by long-term blockade of nitric oxide synthesis in rats. *J Clin Invest*. 1997;99: 278–287.
21. Schieffer B, Wirger A, Meybrunn M, Seitz S, Holtz J, Riede UN, Drexler H. Comparative effects of angiotensin-converting enzyme inhibition and angiotensin II type 1 receptor blockade on cardiac remodeling after myocardial infarction in the rat. *Circulation*. 1994; 89:2273–2282.
22. Michel JB, Xu Y, Blot S, Philippe M, Chatellier G. Improved survival in rats administered L-NAME due to converting enzyme inhibition. *J Cardiovasc Pharmacol*. 1996;28:142–148.
23. Rajagopalan S, Laursen JB, Borthayre A, Kurtz S, Keiser J, Haleen S, Giaid A, Harrison DG. Role for endothelin-1 in angiotensin II–mediated hypertension. *Hypertension*. 1997;30:29–34.
24. d'Uscio LV, Shaw S, Barton M, Luscher TF. Losartan but not verapamil inhibits angiotensin II–induced tissue endothelin-1 increase: role

Downloaded from http://ahajournals.org by on September 29, 2022

of blood pressure and endothelial function. *Hypertension*. 1998;31: 1305–1310.
25. Casellas D, Bouriquet N, Herizi A. Bosentan prevents preglomerular alterations during angiotensin II hypertension. *Hypertension*. 1997; 30:1613–1620.
26. Clavel AL, Mattingly MT, Stevens TL, Nir A, Wright S, Aarhus LL, Heublein DM, Burnett JC. Angiotensin-converting enzyme inhibition modulates endogenous endothelin in chronic canine thoracic inferior vena caval constriction. *J Clin Invest*. 1996;97:1286–1292.
27. Largo R, Gomez-Garre D, Liu XH, Alonso J, Blanco J, Plaza JJ, Egido J. Endothelin-1 upregulation in the kidney of uninephrectomized spontaneously hypertensive rats and its modification by the angiotensin-converting enzyme inhibitor quinapril. *Hypertension*. 1997;29: 1178–1185.
28. Lariviere R, Lebel M, Kingma I, Grose JH, Boucher D. Effects of losartan and captopril on endothelin-1 production in blood vessels and glomeruli in rats with reduced renal mass. *Am J Hypertens*. 1998;11: 989–997.
29. Cottone S, Vadala A, Vella MC, Nardi E, Mule G, Contorno A, Riccobene R, Cerasola G. Changes of plasma endothelin and growth factor levels, and of left ventricular mass, after chronic AT1-receptor blockade in human hypertension. *Am J Hypertens*. 1998;11:548–553.
30. Border WA, Noble NA, Yamamoto T, Harper JR, Yamaguchi Y, Pierschbacher MD, Ruoslahti E. Natural inhibitor of transforming growth factor-β protects against scarring in experimental kidney disease. *Nature*. 1992;360:361–364.
31. Tomita H, Egashira K, Ohara Y, Takemoto M, Koyanagi M, Katoh M, Yamamoto H, Tamaki K, Shimokawa H, Takeshita A. Early induction of TGF-β via angiotensin II type 1 receptors contributes to cardiac fibrosis induced by long-term blockade of nitric oxide synthesis in rats. *Hypertension*. 1998;32:273–279.
32. Sütsch G, Kiowski W, Yan X, Hunziker P, Christen S, Strobel W, Kim J, Rickenbacher P, Bertel O. Short-term oral endothelin-receptor antagonist therapy in conventionally treated patients with symptomatic severe chronic heart failure. *Circulation*. 1998;98:2262–2268.

Downloaded from http://ahajournals.org by on September 29, 2022