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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 [procol α_2 (I)]. Hypertension was induced by chronic inhibition of NO synthesis (*N*^G-nitro-L-arginine methyl ester, L-NAME). Procol α_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 AT₁ receptor antagonist, given simultaneously with L-NAME prevented the increase of procol α_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 procol α_2 (I) was investigated in ex vivo and short-term in vivo experiments. In both conditions, the Ang II-induced activation of procol α_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

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 [procol α_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

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assessed by use of pharmacological blockade of Ang II type I receptors (AT₁) 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 Crombrughe (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 · d⁻¹). In a separate group of control or L-NAME-treated mice, a low dose of losartan (AT₁ antagonist) was administered orally (10 mg · kg⁻¹ · 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 μ g protein (LU/ μ g).

Measurement of Blood Pressure

Systolic blood pressure was measured by the tail-cuff method adapted to the mouse as previously described¹¹ with a piezoelectric sensor (Sensoron 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 μ m 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 (Bio-medica).¹³ Values were expressed as pg endothelin/ μ mol 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 \pm 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 Procol α_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 procol α_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).

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 AT₁ Antagonism on Procol α_2 (I) Gene Activation

AT₁ 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 procol α_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 procol α_2 (I) was also observed in aorta and heart in the late phase of hypertension (14 weeks, Figure 4).

Antagonism of AT₁ 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 Procol α_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

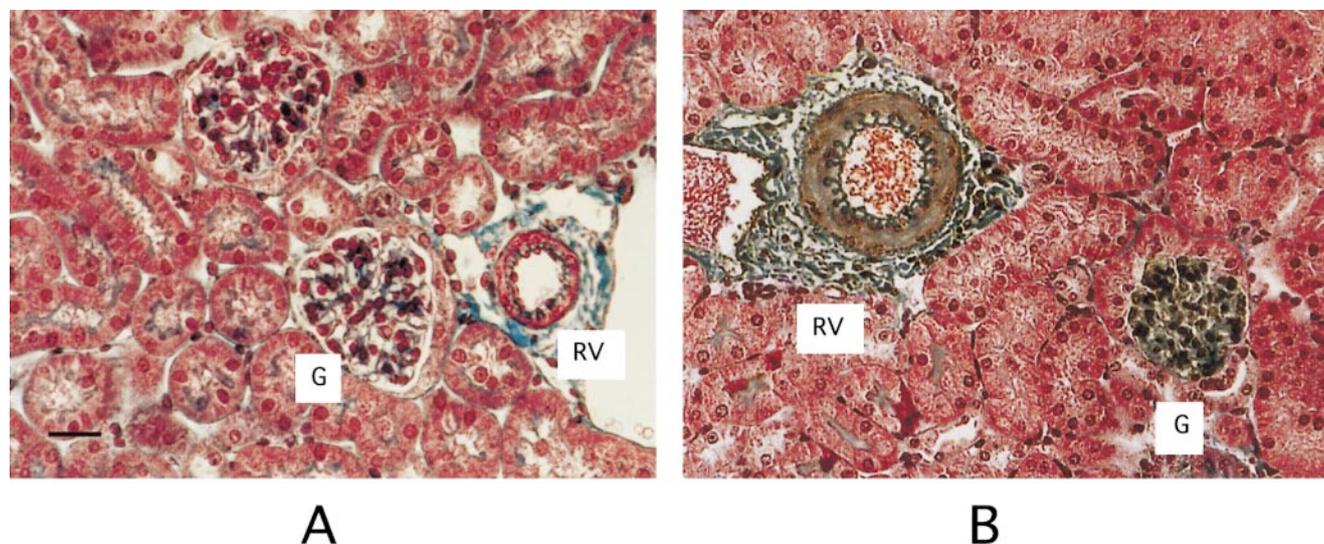


Figure 1. Extracellular matrix staining by Masson's trichrome solution in mouse treated with L-NAME for 6 (A) and 14 (B) weeks. Note normal aspect of glomerulus (G) and renal microvessel (RV) at 6 weeks and intense green staining in these tissues at 14 weeks. Bar=10 μm.

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 procol α_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 procol α_2 (I) activation (Figure 6, top).

Ang II–Induced Activation of the Procol α_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

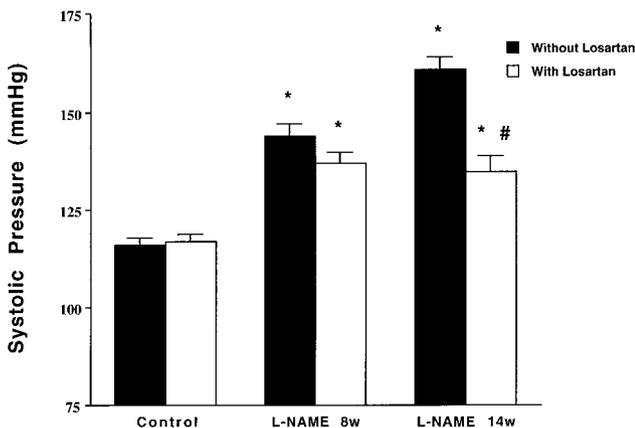


Figure 2. Systolic blood pressure measured in controls and mice treated with losartan, L-NAME, and L-NAME+losartan for 8 or 14 weeks. Values are mean±SEM of 16 mice per group. * $P < 0.05$ vs control; # $P < 0.05$ L-NAME vs L-NAME+losartan-treated group.

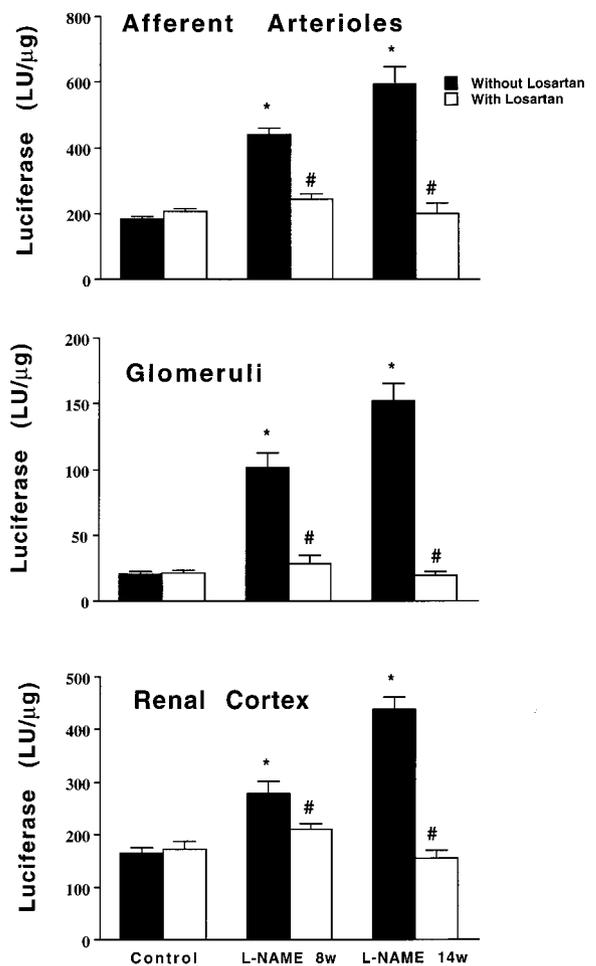


Figure 3. Luciferase activity from afferent arterioles (top), glomeruli (middle), and renal cortical slices (bottom) isolated from controls and mice treated with losartan, L-NAME, and L-NAME+losartan for 8 or 14 weeks. Values are mean±SEM of 16 experiments. * $P < 0.05$ vs control; # $P < 0.05$ L-NAME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

410 ± 35 LU/ μ g 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.

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 procol α_2 (I) and that AT $_1$ antagonism abolished this activation of procol α_2 (I). The capability of Ang II to activate collagen I gene in the renal tissue was corrob-

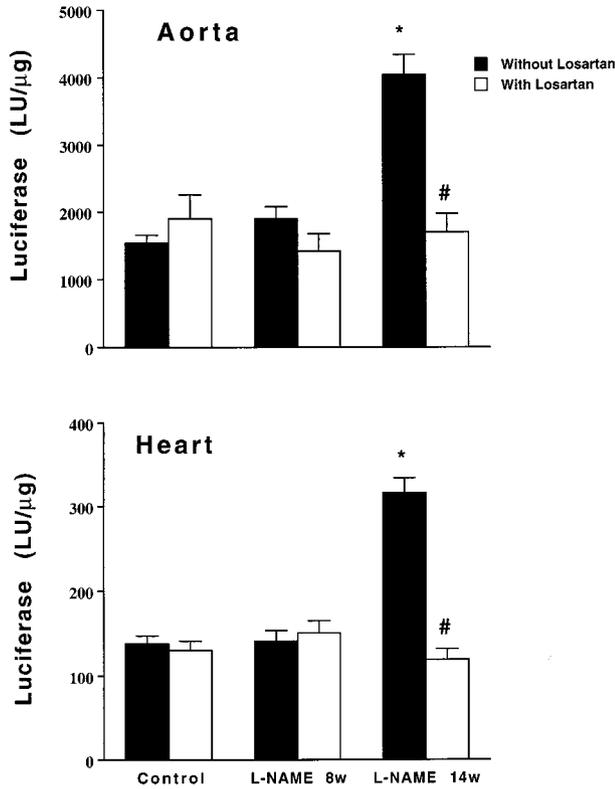


Figure 4. Luciferase activity from aorta (top) and heart (bottom) isolated from controls and mice treated with losartan, L-NAME, and L-NAME+losartan for 8 or 14 weeks. Values are mean \pm SEM of 16 experiments. * P <0.05 vs control; # P <0.05 L-NAME vs L-NAME+losartan-treated group.

orated by ex vivo and acute in vivo experiments. Interestingly, the Ang II-induced activation of procol α_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

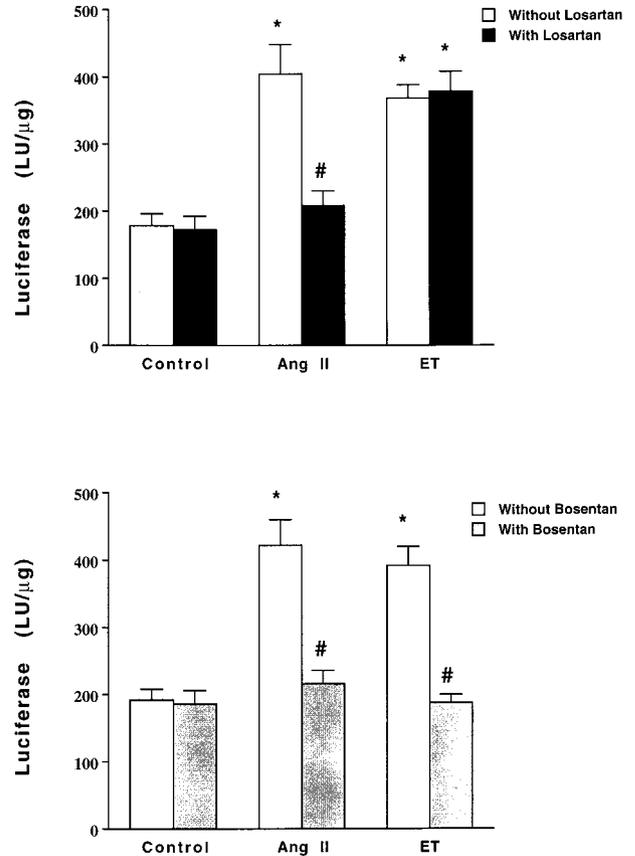
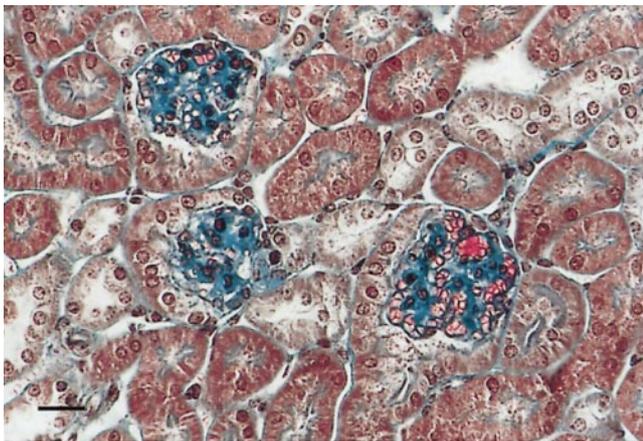
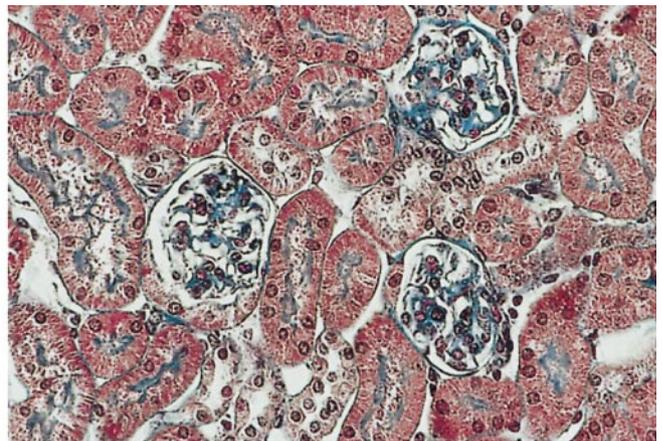


Figure 6. Luciferase activity in renal cortical slices under control conditions and in presence of Ang II or endothelin with or without losartan (top) or bosentan (bottom). Values are mean \pm SEM of 10 experiments. * P <0.05 vs control; # P <0.05 vs Ang II or endothelin.

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



A



B

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 μ m.

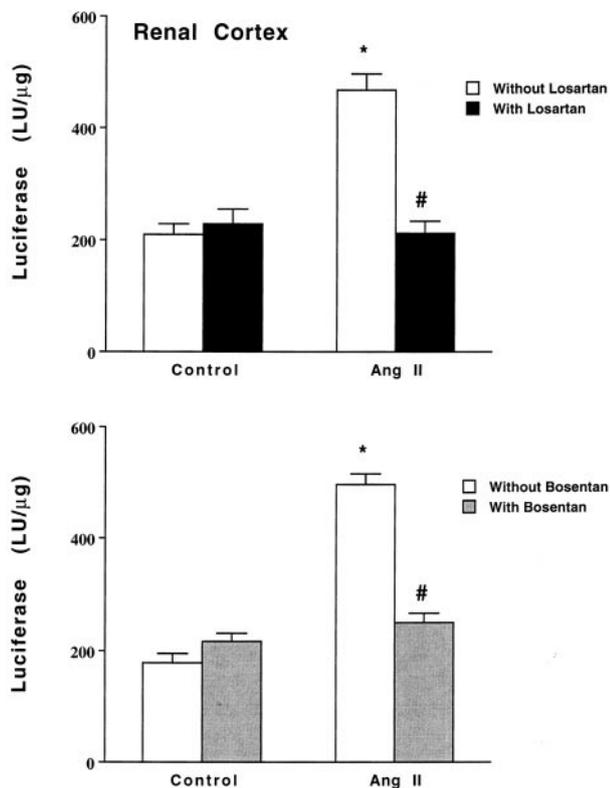


Figure 7. Effect of AT₁ and endothelin antagonism on Ang II-induced collagen I gene activation in renal cortical slices in vivo. Values are mean \pm SEM of 12 experiments. * $P < 0.05$ vs control; # $P < 0.05$ vs Ang II-injected group.

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 AT₁ antagonists markedly reduced the development of L-NAME-induced cardiac and renal fibrosis and improved survival.^{20–22} In agreement with these studies, AT₁ 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, AT₁ antagonism normalized procol α_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 Procol α_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 procol α_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 AT₁ 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 procol α_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 procol α_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 (5% 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 AT₁ 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

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 AT₁ 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. AT₁ 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 AT₁ 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 AT₁ 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.

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