Losartan increases NO release in afferent arterioles during regression of L-NAME-induced renal damage

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Inhibition of nitric oxide synthase (NOS) induces hypertension and heavy proteinuria. Renal structure and function have shown striking improvement after interventions targeting ANG II or endothelin (ET) receptors in rats recovering after long-term NOS inhibition. To search for mechanisms underlying losartan-assisted regression of renal disease in rodents, we measured NO release and contractility to ET in afferent arterioles (AAs) from Sprague-Dawley rats recovering for 2 wk after 4 wk of N\textsuperscript{G}-nitro-L-arginine methyl ester treatment. Losartan administration during the recovery period decreased blood pressure (113 ± 4 vs. 146 ± 5 mmHg, P < 0.01), reduced protein/creatinine ratio more (proteinuria decrease: Δ1.836 ± 214 vs. Δ1.024 ± 180 mg/mmol, P < 0.01), and normalized microvascular hypertrophy (AA media/lumen ratio: 1.74 ± 0.05 vs. 2.09 ± 0.08, P < 0.05) compared with no treatment. In diaminofluorescein-FM-loaded AAs from losartan-treated animals, NO release (% of baseline) was increased compared with untreated animals after stimulation with 10\textsuperscript{-7} M ACh (118 ± 4 vs. 90 ± 7%, t = 560 s, P < 0.001) and 10\textsuperscript{-9} M ET (123 ± 4 vs. 101 ± 5%, t = 560 s, P < 0.001). There was also a blunted contractile response to 10\textsuperscript{-7} M ET in AAs from losartan-treated animals compared with untreated animals (Δ4.01 ± 2.9 vs. Δ1.46 ± 1.7 μm, P < 0.01), which disappeared after acute NOS inhibition (Δ1.07 ± 3.7 vs. Δ12.5 ± 2.9 μm, P > 0.05). Contractile dose responses to ET (10\textsuperscript{-10}, 10\textsuperscript{-8}, 10\textsuperscript{-7} M) were enhanced by NOS inhibition and blunted by exogenous NO (10\textsuperscript{-2} mM S-nitroso-N-acetyl-penicillamine) in losartan-treated but not in untreated vessels. Reducing blood pressure similar to losartan with hydralazine did not improve AA hypertrophy, ET-induced contractility, ET-induced NO release, and NO sensitivity. In conclusion, blockade of the local action of ANG II improved endothelial function in AAs, which is a mechanism that is likely to contribute to the beneficial effects of AT\textsubscript{1}R antagonism during the recovery of renal function after long-term NOS inhibition in rats.

endothelin; nitric oxide; endothelial dysfunction; angiotensin II; vascular remodeling

deterioration of renal function is a result of several mechanisms, such as disturbances of renal blood flow (RBF), endothelial dysfunction, inflammation, fibrosis, or increased deposition of extracellular matrix. At present, few therapeutic tools are available to halt these processes, and the final result is often chronic renal failure. The only available treatments for these patients are dialysis or renal transplantation, costly replacement therapies that are available only for a limited portion of the world's population. An important challenge in modern medicine is therefore the development of intervention strategies that may halt or reverse the development of chronic renal failure.

Drugs that are able to suppress the renin-angiotensin system have shown remarkable results in reversal of chronic renal disease in rodents (1, 3). In contrast, large-scale human trials showed less effect of AT\textsubscript{1}R antagonism, but a slower progression of renal disease has been found (13, 17). A major focus of the present study was to explore how the beneficial effects of losartan, an AT\textsubscript{1}R blocker, are mediated in the rodent kidney, to gain insight into mechanisms of AT\textsubscript{1}R antagonism in regression of renal disease.

Inhibition of nitric oxide (NO) synthase (NOS) by N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) is a well-documented model of experimental hypertension that has been used to study the progression of renal pathology. This model has been further developed at our laboratory to study renal regression after a predetermined loss of renal function, measured as protein/creatinine ratio.

In a recent study, we examined animals recovering after 4-wk NOS inhibition and found RBF to be improved in animals treated with losartan during the regression period compared with animals receiving water only (8). The improvement of renal hemodynamics was associated with improved morphological structure and reduced inflammation in the kidney. The exact mechanisms how AT\textsubscript{1}R inhibition confers these favorable changes are not clear. In contrast to our previous experiments (3), L-NAME was removed from the drinking water when applying the intervention (losartan) in the present experiment. Thus, regression with AT\textsubscript{1}R antagonism was compared with rats recovering without continuous pharmacological NO denial.

The afferent arterioles (AAs) represent an important resistance segment in the kidney (16) that protects glomeruli from pressure variations, and thereby counteract development of renal glomerular injury and proteinuria. The AAs are the most responsive segment of renal vasculature to bolus injections of ANG II (15), indicating a pivotal role of these vessels during AT\textsubscript{1}R blockade. We therefore analyzed the structural and functional effects of losartan treatment during regression of renal injury in this vascular segment.

The major aim of the present study was to uncover the basic mechanisms of losartan-assisted regression during recovery after L-NAME treatment in rats. Our working hypothesis was that agonist-induced NO release and vasoconstriction are more favorable in AAs from animals treated with losartan compared with animals not treated during recovery after long-term NOS inhibition. To further explore the local vs. the systemic pressure component of AT\textsubscript{1}R antagonism, we also compared

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losartan treatment with hydralazine, a blood pressure-lowering drug that does not interfere with the renin-angiotensin system.

**MATERIALS AND METHODS**

*Animals.* Thirty-seven male Sprague-Dawley rats weighing 250 g at the start of the experiments were fed ordinary rat pellets containing 0.5% sodium, 0.6% potassium, 0.71% calcium, and 14.7% crude protein. The rats had free access to tap water. The experiments were performed in accordance with and under the approval of the Norwegian State Board for Biological Experiments with Living Animals.

*Experimental groups.* The design of the experimental protocol is shown in Fig. 1. During the l-NAME treatment (30 mg·kg$^{-1}$·day$^{-1}$ in drinking water), the general condition of the animals was monitored daily. After 3 wk, urinary protein excretion was monitored once every week, or twice every week if P/C ratio exceeded 500 mg/mmol. Untreated rats were used as controls ($n$ = 9). When proteinuria exceeded 1.5 g/mmol creatine (usually after 4–6 wk), animals receiving l-NAME were divided into four experimental groups. In the first group, animals were killed immediately after reaching 1.5 g/mmol creat (l-NAME group, $n$ = 6). In the second group, l-NAME was removed from the drinking water and animals were kept for 2 wk with no treatment (removal + water group, $n$ = 8). In the third group, l-NAME was removed from drinking water and replaced with losartan (60 mg·kg$^{-1}$·day$^{-1}$) for 2 wk (removal + losartan group, $n$ = 8). In the fourth group, l-NAME was removed from drinking water and replaced with hydralazine (30 mg·kg$^{-1}$·day$^{-1}$) for 2 wk ($n$ = 6).

*Measurement of blood pressure.* Systolic blood pressure was measured after 20–30 min preheating at 32°C by the tail-cuff method using CODA 6 (Kent Scientific) following the instructions of the manufacturer.

*Urinary protein/creatinine ratio.* Protein and creatinine levels were measured using Roche/Hitachi 912: U/CSF Protein and CREA plus essays.

*Isolation and preparation of AAs. AAs (Fig. 2A) were isolated for measurements of NO release and diameter measurements by use of the agarose infusion/enzyme treatment technique originally described by Loutzenhiser and Loutzenhiser (14) and later on adapted and modified in our laboratory (7). In short, the kidneys were infused with the enzymes, free-floating AAs free of connective tissue and with an area

<table>
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<tr>
<th>Start of experiment</th>
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<tr>
<td>No treatment</td>
<td>Death of rats</td>
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<tr>
<td>L-NAME, 30 mg/kg/d</td>
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**Fig. 1.** Experimental protocol. When proteinuria reached 1.5–2 g/mmol (usually after 4–6 wk), N$^\text{G}$-nitro-l-arginine methyl ester (l-NAME) was removed and replaced either with water, water containing the peripheral smooth muscle relaxant hydralazine, or water containing the AT$\text{R}$ antagonist losartan for 2 wk.

*NO fluorescence and mean lumen diameter measurements.* Isolated vessels were attached by self-adhesion to the clean glass surface of a No 1.5 coverslip mounted on a petri dish. Vessels were superfused with 3 ml medium without Ca$^{2+}$. After the experiments, Ca$^{2+}$ concentration in the medium was gradually increased to 2 mmol/l in three steps (20 μmol/l, 200 μmol/l, and 2 mmol/l) with 3- to 5-min incubation in between. Vessels were loaded with 5.0 μmol/l diaminofluorescein-FM (DAF-FMdiacetate at room temperature for 1 h. After wash-out of the dye, vessels were incubated for 15 min at 37°C to ensure complete deesterification, since DAF-FM was added to the perfusion bath as an ester to facilitate passage across the cell membrane.

Using a Leica SP2, confocal bright-field (Fig. 2B) and fluorescence (Fig. 2, C–D, excitation 488 nm, emission >510 nm, 512 × 512 pixels) images were acquired for quantification of mean lumen diameter and NO fluorescence, respectively. Fluorescence images were imported to WCIF ImageJ 1.37c for quantification of background-subtracted DAF-FM fluorescence using the Multi Measure plugin. Lumen area and length were traced from the bright-field images using Olympus DP-Soft 5.0. Mean lumen diameter was calculated using the formula mean lumen diameter = lumen area/lumen length, as exemplified in Fig. 2E (0 s) and 2F (400 s). Analysis of NO fluorescence and diameters was performed every 40 s except for ET dose responses for which diameters were analyzed at baseline and once after 3 min for each successive concentration of the agonist. One or two arterioles were used from each animal in each experiment.

**Assessment of AA hypertrophy.** Before DAF loading, total arteriole and total lumen area during baseline were recorded to calculate vessel wall thickness and media lumen ratio, as a measure of microvascular hypertrophy. Mean vessel wall thickness was calculated as [(arteriole area − lumen area)/2]/arteriole length. Mean lumen diameter was measured using the Multi Measure plugin. Lumen area and length were traced from the bright-field images using Olympus DP-Soft 5.0. Mean lumen diameter was calculated using the formula mean lumen diameter = lumen area/lumen length, as exemplified in Fig. 2E (0 s) and 2F (400 s). Analysis of NO fluorescence and diameters was performed every 40 s except for ET dose responses for which diameters were analyzed at baseline and once after 3 min for each successive concentration of the agonist. One or two arterioles were used from each animal in each experiment.

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<td>Removal + Water</td>
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<tr>
<td>Removal + Hydralazine</td>
<td>2 wk, $n$ = 6</td>
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<tr>
<td>Removal + Losartan</td>
<td>2 wk, $n$ = 8</td>
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calculated as lumen area/lumen length. Media/lumen ratio was calculated as media area/lumen area. Three arterioles were used from each animal.

**Chemicals.** The buffer consisted of 135 mmol/l NaCl, 5.00 mmol/l KCl, 1.00 mmol/l MgSO4, 2.40 mmol/l CaCl2, 10.0 mmol/l glucose, 2.60 mmol/l Na2HPO4, 10% HEPES, and 1.15 mmol/l L-arginine. All chemicals were bought from Sigma.

**Statistical methods.** All statistical tests were performed using one-way ANOVA followed by the Student-Newman-Keuls post hoc test in SigmaStat 3.1. Kaplan-Meyer log rank test was used to analyze survival in the three recovery groups and performed in Sigma Stat 3.1. P < 0.05 was considered statistically significant. All values are means ± SE.

**RESULTS**

Animals given losartan changed their behavior and appearance compared with rats not taking this drug. They resembled control rats, moved normally in their cages, had clean fur, and had normal body tonus when handled. In sharp contrast, animals from the removal + losartan group and removal + hydralazine groups were less active, had ragged fur, and displayed reduced body tonus when handled. In addition, both of the two latter groups suffered from mortality. Using a Kaplan-Meyer log rank analysis, survival was found to be significantly reduced in
the untreated group or rats treated with hydralazine compared with losartan treatment (P = 0.03).

Table 1 shows that mean arterial pressure (MAP) decreased from 169 ± 8 to 146 ± 5 mmHg [not significant (NS)] during unassisted recovery (removal + water group). Losartan or hydralazine administration reduced MAP to 113 ± 4 and 112 ± 7 mmHg, respectively (P < 0.01 vs. l-NAME group, NS vs. controls). Following removal of l-NAME, proteinuria improved significantly and almost twice as much in losartan compared with nontreated or hydralazine-treated rats (P < 0.05). AT1aR blockade reduced media-to-lumen ratio to normal levels. In contrast, removal of l-NAME combined with water or hydralazine administration did not improve AA structure.

In a recent study, we observed little or no contractile response to 10−7 M ANG II in losartan-treated animals (9), probably due to the high affinity between losartan and the AT1aR. We therefore had to use another vasoactive hormone to assess microvascular function in the kidney. ET has previously been shown to induce exaggerated vasoconstriction and cause renal damage in l-NAME-treated rodents (2, 4, 20) and has no affinity to the AT1aR. Due to these reasons, the vascular response to ET was used as a measure of renal microvascular function.

A major goal of the present study was to measure agonist-induced NO release directly in vessels loaded with the NO-specific dye DAF-FM. ACh induced an early increase in NO-derived fluorescence in vessels from losartan-treated animals, which was not seen in the other groups (n = 10–12, P < 0.05; Fig. 3A). Stimulation with a near physiological dose with ET (10−9 M) induced a steep increase in NO release in losartan-treated animals, whereas AAs from the other groups did not respond (n = 7–10, P < 0.05; Fig. 3B).

Simultaneously with the quantification of NO fluorescence, bright-field images were acquired to measure diameter changes. AAs from the l-NAME, removal + water, and removal + hydralazine group showed a significant decrease in diameter after 10−9 M ET administration (n = 9–10; Fig. 3, C-D). In contrast, when vessels from the control and removal + losartan group were given 10−9 M ET, the diameter of AAs did not change.

To further explore the dependency of vasodilatory NO release during ET stimulation, vessels were stimulated with or without 10−4 M l-NAME in the vessel bath. Consistent with previous reports (2), pilot experiments demonstrated large variations in the contractile sensitivity to 10−9 M ET, which made it difficult to interpret the effects of l-NAME administration. Applying a saturating dose of ET (10−7 M), however, produced more uniform responses and made it possible to compare the effect of acute NOS inhibition among the groups.

As seen in Fig. 4, A and C (n = 8–9), there was a blunted response in vessels from the removal + losartan group that disappeared if vessels were pretreated with l-NAME (n = 8; Fig. 4, B and D). Vessels from the remaining groups displayed a strong response to ET, regardless if l-NAME was present or not in the medium.

To better analyze the dose-response sensitivity to ET, concentrations from 10−10 to 10−7 M ET were administered to vessels from the removal + water, removal + hydralazine, or removal + losartan in the presence of 10−4 M l-NAME with or without exogenous supplied NO (10−2 mM SNAP; Fig. 5). In removal + water or removal + hydralazine groups, NOS inhibition did not have any additional effect on arteriole diameter regardless of what concentrations of ET were used. In addition, vessels from water (Fig. 5, A and D) and hydralazine-treated (Fig. 5, B and E) animals did not display blunted responses to ET when the NO donor SNAP was present in the medium. On the other hand, starting at 10−5 M ET, the contractile response of losartan-treated vessels was stronger if l-NAME was present in the vessel bath (P < 0.05). Consistent with these data, the diameter response to ET was blunted by exogenous NO starting at 10−10 M compared with l-NAME only (P < 0.05; Fig. 5, C and F). Moreover, these vessels displayed improved endothelial function, since administration of SNAP abolished the contractile effect of l-NAME.

**DISCUSSION**

The present study provides new information about the mechanism of losartan-assisted regression of renal function in rats recovering from long-term NOS deficiency. AT1aR inhibition increased agonist-induced NO release, blunted the contractile response to ET, and increased vascular sensitivity to NO. These changes were associated with reduced microvascular hypertrophy and proteinuria. Thus, increased vasodilatory NO release and AT1aR antagonism are likely to have induced beneficial and additive effects on renal circulation during recovery after long-time NOS inhibition.

Consistent with previous studies (3), losartan treatment reduced MAP, improved proteinuria and afferent arteriolar hypertrophy compared with animals receiving water only (Table 1). In contrast to these studies, however, l-NAME was removed from the drinking water during the recovery period in the present protocol. It may therefore be concluded that losartan has a beneficial effect on blood pressure and proteinuria not only during continuous NOS denial, as shown previously, but also during regression without continued l-NAME treatment.

**Table 1. MAP, proteinuria, lumen diameter, vessel wall thickness, and media/lumen ratio**

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mmHg</th>
<th>Proteinuria Decrease, mg/mmol</th>
<th>Lumen Diameter, μm</th>
<th>Vessel Wall, μm</th>
<th>Media/Lumen Ratio</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>99 ± 8</td>
<td></td>
<td>21.0 ± 1.4</td>
<td>8.5 ± 0.6</td>
<td>1.80 ± 0.07</td>
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<tr>
<td>l-NAME</td>
<td>169 ± 8*</td>
<td></td>
<td>22.3 ± 1.4</td>
<td>13.1 ± 0.8*</td>
<td>2.26 ± 0.12*</td>
</tr>
<tr>
<td>Removal + water</td>
<td>146 ± 5*</td>
<td></td>
<td>23.1 ± 1.2</td>
<td>12.1 ± 0.6*</td>
<td>2.09 ± 0.08*</td>
</tr>
<tr>
<td>Removal + hydralazine</td>
<td>112 ± 7*</td>
<td></td>
<td>19.3 ± 1.6</td>
<td>11.1 ± 1.7</td>
<td>2.44 ± 0.14*</td>
</tr>
<tr>
<td>Removal + losartan</td>
<td>112 ± 4*</td>
<td></td>
<td>25.3 ± 1.0*</td>
<td>9.0 ± 0.4</td>
<td>1.74 ± 0.05</td>
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Values are means ± SE. Mean arterial pressure (MAP), improvement of proteinuria, lumen diameter, vessel wall thickness, and media/lumen ratio in rats from control (n = 9), Nω-nitro-l-arginine methyl ester (l-NAME; n = 6), removal + water (n = 8), removal + hydralazine (n = 6), and removal + losartan (n = 8) groups. *P < 0.001 vs. control. ** And ***P < 0.05 and P < 0.005, respectively, vs. control and removal + losartan. ^And *P < 0.05 and P < 0.001, respectively, vs. l-NAME and removal + water. #P < 0.01 vs. removal + water and removal + hydralazine. #P < 0.05 vs. removal + hydralazine.
Two weeks after cessation of l-NAME treatment, blood pressure, proteinuria, and AA hypertrophy were still unfavorable in animals not receiving losartan treatment. The reason for this is not clear, but it might be due to longstanding effects of the NOS inhibition that persists after removal of L-NAME. In this respect, it is interesting to note that losartan was even more efficient in normalizing blood pressure and proteinuria compared with intervening with losartan during continued administration of L-NAME (3).

Blood pressure was substantially reduced in losartan-treated animals, and one might question whether the improved renal function and structure resulted from the normalization of blood pressure alone, or if AT1aR antagonism had additional effects on renal vasculature independent of systemic pressure. Our present results with the hydralazine group agree with the latter hypothesis. Although hydralazine reduced systemic pressure similar to losartan levels, it failed to improve contractility, microvascular hypertrophy, proteinuria, and mortality. This indicates that losartan mediated beneficial effects on renal structure and function that cannot be explained by a blood pressure-lowering effect alone. This observation is in agreement with our previous results in which AT1R blockade, but not hydralazine, inhibited fibrillar collagen and TGF-β synthesis and reduced renal fibrosis and glomerulosclerosis despite a similar effect on blood pressure with these treatments (3).

NO generated by endothelial (e)NOS is triggered by two stimuli in the afferent arteriolar vasculature: shear stress-dependent continuous release (19) and agonist-induced release independent of physiological stimuli (18). In experiments with perfused arterioles, application of a simple contractile stimulus that does not induce NO by itself, such as 100 mM KCl, may still alter flow and consequently shear stress, due to the reduced diameter of the vessel. With agonists such as ET, this may complicate the interpretation of the response, since it is difficult to know whether NO release is the result of altered physiological parameters in the arterioles, induced directly by the agonist, or a combination of both. To circumvent this problem, we used an agarose-infused preparation of the AA, which permits simultaneous NO and diameter measurements, but is devoid of flow. This allowed us to study the agonist-induced component of NO release alone, without interference from other physiological parameters such as shear stress. Diameter measurements in this preparation are possible due to the elasticity of the intravascular agarose (Fig. 2A, arrowhead), which is compressed during contraction of the vessel (Fig. 2, E–F).

To test the general capacity of NO release, ACh was used to directly induce release of this hormone (22). ACh induced a significant increase in NO fluorescence in AAs from losartan-treated animals, indicating that AT1aR blockade ameliorated endothelial function of renal resistance vessels. It is well-established that ACh induces vasodilation in a dose-dependent manner in preconstricted AAs (21, 22), and it has been assumed that this effect is always mediated by NO. It may therefore seem inconsistent that NO fluorescence from control AAs was unchanged after ACh stimulation in the
present experiment. It is, however, not clear that NO is the mediator of ACh-induced vasodilation during control conditions in the AA. Garland et al. (6) suggested that endothelial-derived hyperpolarizing factor, rather than NO, is more important for control of vascular tone in small blood vessels. Kimura et al. (10) found that ACh induced less NO in cultured endothelial cells from microvessels, compared with cultured endothelial cells of aortic origin. Drouin et al. (5) found in mice that ACh-induced NOS-dependent vasodilation was mediated by H$_2$O$_2$ in cerebral arteries and by NO in gracilis arteries, suggesting different enzyme products of NOS in different vascular beds. To our knowledge, the present study is the first to quantify the NO response to ACh directly in the AA ex vivo using intracellular dyes, and the tracings indicate that the NO response induced by ACh during no-flow conditions is either below the detectable level with DAF-FM [5 nM (11)], or mediated by another messenger, such as H$_2$O$_2$.

DAF-FM fluorescence in AAs from losartan-treated animals was increased after application of a near physiological dose of ET [10$^{-9}$ M, resting levels are $\sim$10$^{-12}$ M (23)], indicating that not only the general capacity of NO release, as indicated by the ACh response, but also the specific NO response to ET was increased after AT$_1$R antagonism (Fig. 3B). Increased vasoactivity to ET has been suggested to constitute a major component of the blood pressure increase during t-NNAME-induced hypertension in the rat (2), and an enhancedvasodilatory action by NO may counter this effect. In the remaining groups, DAF-FM fluorescence was unchanged after ET, indicating that NO release was absent or below detectable levels. Lumen diameter did not change after administration of 10$^{-9}$ M ET in AAs from control and losartan-treated animals, indicating a normalized response to this hormone in treated animals. In contrast, AAs from the other groups had a considerable contractile response to 10$^{-9}$ M ET that would have had a substantial impact on renal circulation in vivo (Fig. 3, C and D). Consistent with endothelial dysfunction-associated structural abnormalities, AAs from L-NAME, removal + water, and removal + hydralazine animals had a hypertrophied vessel wall. Only when endothelial dysfunction was corrected with AT$_1$R antagonism was vessel structure improved and similar to control. Although AAs from both control and losartan-treated animals had low contractile sensitivity to ET, NO fluorescence induced by this hormone was increased only in AAs from the latter group. Thus, renal microvessels may have been sensitized to ET in all groups receiving t-NNAME, but counteracted by NO release only in the animals treated with losartan. This also implies that losartan did not normalize NO release in the present experiment. On the contrary, AT$_1$R inhibition seems to have upregulated agonist-induced NO release to higher than control levels. By this mechanism, a normalized response to 10$^{-9}$ M ET was found in AAs from losartan-treated animals, but not in vessels from the remaining groups, which lacked this vasodilatory response.

To evaluate more closely the functional impact of NO release in losartan-treated animals, we applied a saturating dose of ET (10$^{-7}$ M) that ensured a maximum signal for both contraction (ET$_A$ receptors) and NO release (ET$_B$ receptors) (24) with or without acute NOS inhibition. In groups not
treated with losartan, vessels contracted to ~40% of baseline after ET stimulation. In AAs from losartan-treated animals, however, there was a blunted contractile response to ET (~80% of baseline diameter), which became stronger and similar to the other groups if NO release was inhibited ex vivo with L-NAME (Fig. 4). This evidence further supports the notion that the contractile response to ET was buffered by NO release in losartan-treated animals.

Further dose-response studies indicated NOS-dependent vasodilation at ET concentrations from $10^{-9}$ to $10^{-7}$ M ET in losartan-treated vessels, suggesting that this vasodilatory response is active somewhere between 100 and $10^3$ times the resting level of ET (~$10^{13}$ M; Fig. 5) (23). At none of the ET doses did vessels from the removal + water (A, D) and removal + hydralazine groups (B, E) have a significantly increased diameter response to ET during NOS inhibition, which did not affect the contractile response to ET. Losartan-treated vessels (C, F) had a significantly increased diameter response to ET during NOS inhibition, which became normal if exogenous NO was added to the vessel bath in addition to L-NAME. * and ** P < 0.05 and P < 0.01 vs. normal medium. † and †† P < 0.05 and P < 0.001 vs. acute NOS inhibition.

NO is reported to be induced by AT$_{1a}$R stimulation (18), and it may seem a paradox that inhibition of this receptor with losartan also upregulates agonist-induced NO release. We have in fact found no studies supporting the notion that losartan directly induces NO release. Losartan is, however, hydrolyzed into two metabolites after entering the bloodstream: EXP 3174, which has an AT$_{1a}$R-blocking effect, and EXP 3179, which has no effect on the receptor (12). The latter metabolite, EXP 3179, induced phosphorylation of eNOS at ser 1179 (which increases activity of the enzyme) and its upstream activator Akt (protein kinase B) in aortic bovine endothelial cell culture (25). This finding provides a possible explanation for the increased NO release seen in losartan-treated animals in the present experiment. Additional investigation is necessary, however, to see whether EXP 3179 has the same effect in renal microvessels.

Interestingly, a similar response to losartan was observed in arteries from hindlimb unweighted rats, a model of microgravity exposure. During this special type of cardiovascular stress, losartan restored the general vasoreactivity, ACh-induced vasorelaxation, and increased eNOS protein expression (26), indicating improved NOS function.

In conclusion, the present study provides new insight in the mechanisms by which AT$_{1a}$R antagonism assists regression of kidney disease. Contrary to what we expected, losartan did not just normalize ET-induced NO release, but increased the NO response to higher than control levels. Consistent with this, there was a blunted contractile response to ET in AAs from losartan-treated animals, which became stronger if NO was...
blocked ex vivo. The present data demonstrate that blockade of the AT1a receptor induces vascular protection by improving endothelial function, regressing vascular hypertrophy and correcting microvessel function and structure, in addition to previously reported anti-fibrotic action. These observations, if confirmed in humans, will provide hope that chronic kidney disease can be efficiently treated.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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