Renal expression of COX-2, ANG II, and AT1 receptor in remnant kidney: strong renoprotection by therapy with losartan and a nonsteroidal anti-inflammatory

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doids through hemodynamic and inflammatory mechanisms and atten
duated by individual suppression of these mediators. In rats with 50% renal ablation (Nx), we investigated 1) the intrarenal distribution of COX-2, ANG II, and the AT1 receptor (AT1;R); 2) the renoprotective and antiinflammatory effects of an association between the AT1;R blocker, losartan (Los), and the gastric sparing anti-inflammatory nitrofurans (NOF). Adult male Munich-Wistar rats underwent Nx or sham operation (S), remaining untreated for 30 days, after which renal structure was examined in 12 Nx rats (Nxpre). The remaining rats were followed during an additional 90 days, distributed among 4 treatment groups: NxV (vehicle), NxLos (Los),NxNOF (NOF), and NxLos/NOF (Los/NOF). Nxpre rats exhibited marked albuminuria, hypertension, glomerulosclerosis, interstitial expansion, and macrophage infiltration, accompanied by abnormal glomerular, vascular, and interstitial COX-2 expression. ANG II appeared in interstitial cells, in contrast to S, in which ANG II was virtually confined to afferrent arterioles. Intrarenal AT1;R distribution shifted from mostly tubular in S to predominantly interstitial in Nxpre. All these changes were aggravated at 120 days and attenuated by Los and NOF mono
therapies. Los/NOF treatment arrested renal structural injury and ANG II expression and reversed hypertension, albuminuria, and renal inflammation. In conclusion, abnormal expression of COX-2, ANG II, and AT1;R may be key to development of renal injury in Nx. Con
comitant COX-2 inhibition and AT1;R blockade arrested renal injury and may represent a useful strategy in the treatment of chronic nephropathies.

prostaglandin-endoperoxide synthase; angiotensin II; anti-inflamma
tory agents; kidney failure; chronic; inflammation

BOTH HEMODYNAMIC AND INFLAMMATORY factors are involved in the pathogenesis of chronic progressive nephropathies, whereas angiotensin II (ANG II) and prostanoi
doids are thought to participate in both kinds of events. Accordingly, cyclooxy
genase inhibitors, and especially suppressors of the renin
angiotensin-aldosterone system (RAS), were shown to retard the progression of chronic experimental nephropathies. RAS inhibitors have also been successful in the treatment of human nephropathies (3, 22), suggesting that ANG II plays a central pathogenic role in these processes.

The beneficial effect of RAS suppressors was initially attributed to amelioration of the glomerular hemodynamic dys
tunction associated with progressive nephropathies. However, recent observations suggest that the nonhemodynamic effects of RAS suppressors may be equally important, given the strong proinflammatory and profibrotic effects of ANG II (35). A substantial fraction of this proinflammatory ANG II may orig
inate in the renal parenchyma, rather than in renal vessels or in the systemic circulation (44). Increased intrarenal production of ANG II was described in various models of renal fibrosis (12, 30, 34). A preliminary report has suggested that, in the 50% renal ablation (Nx) model, ANG II is expressed in renal interstitial cells, paralleling the severity of renal injury (28).

Both the hemodynamic and proinflammatory effects of ANG II are mediated by AT-1 receptors (AT1;R) (35), extensively expressed in renal tissue. In the normal rat kidney, AT1;R are predominantly expressed in tubular cells and vessels (15). Recent data obtained with the Nx model have suggested that AT1;R expression is shifted from the glomerular to the tubulo-interstitial compartment 4 wk after ablation (6). However, the renal distribution of AT1;R in this model and its temporal evolution have not been established.

Cyclooxygenase (COX) derivatives may play an important role in the pathogenesis of progressive nephropathies, compara
tible to their role in arthritis. Increased renal expression of isoforms 1 and 2 of COX has been reported in nephropathies of immunological and nonimmunological origin, such as systemic lupus erythematosus (43), glomerulosclerosis in Fawn-Hooded hypertensive rats (50), Heymann nephritis (40), and renal ablation (9, 49). In a recent study by this laboratory, increased renal expression of COX-2, which correlated significantly with the extent of renal damage, was shown in Nx rats (9). In addition, chronic use of COX inhibitors greatly attenuated renal injury in the Nx model (9, 10, 48).

In view of the complexity and the large number of events leading to progressive renal fibrosis, the interruption of two or more pathogenic pathways by a combination of drugs with different mechanisms of action is likely to be more effective than the respective monotherapies. Three independent studies showed that the combination of a RAS suppressor with an immuno
suppressive agent exerted a much stronger protective effect on Nx rats than either drug alone (11, 13, 33).

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In the present study, we evaluated in Nx rats the renal distribution of both the AT1R and the COX-2 isomorph, as well as the variation of their renal expression with time. In addition, we estimated the amount of ANG II present in glomerular arterioles and in the cortical interstitium. To evaluate the role of these mediators in progression, Nx rats were treated with nitrofuribuprofen (NOF), a nonsteroidal anti-inflammatory drug (NSAID) with low gastrointestinal toxicity, or losartan potassium (Los), an AT1R blocker. In an attempt to obtain more effective renal protection, a group of Nx rats receiving a combined NOF/Los treatment was studied as well.

METHODS

Seventy-seven adult male Munich-Wistar rats, obtained from a local breeding colony, with initial weights of 240 to 260 g, were used in this study. Under anesthesia with pentobarbital sodium (50 mg/kg ip), nephrectomy was performed by removal of the right kidney and ligation of the appropriate left renal artery branches, thus ensuring the infarction of at least two-thirds of the left kidney. Twelve sham-operated (S) rats underwent anesthesia, ventral laparatomy, and manipulation of the renal pedicles, without any removal of renal mass. After recovering from anesthesia, the animals were returned to their original cages, given free access to tap water and standard chow (0.5% Na, 22% protein), and maintained at 23 ± 1 °C under a 12:12-h light-dark cycle. All experimental procedures were in strict accordance with our institutional guidelines and with international standards for manipulation and care of laboratory animals, being previously approved by the local Research Ethics Committee.

Experimental groups. Thirty days after Nx, the tail-cuff pressure (TCP) was measured by an indirect method (11). The animals were then placed in metabolic cages for determination of daily urinary albumin excretion rate (UalbV). Animals that at this time had failed to develop hypertension (defined as TCP ≥140 mmHg) or albuminuria (UalbV ≥50 mg/day) were excluded from the study. The kidneys of 12 Nx rats were then perfusion-fixed with Duboscq-Brazil solution (0.45% picric acid in a mixture of ethanol, formaldehyde, and acetic acid) after a brief saline washout and prepared for light microscopic and immunohistochemical analysis as described below. This group, designated Nxpre, was used to evaluate the extent of renal injury at 30 days afterNx and served to assess the therapeutic efficacy of treatments started thereafter. The remaining Nx rats were then followed for an additional 3 mo (up to 4 mo after nephrectomy) after having been distributed among four experimental groups: Nx (n = 12), Nx rats receiving inert vehicle; NxNOF (n = 14), Nx rats receiving NOF (Nicox, Sophia Antipolis), 7.5 mg/kg dissolved in a mixture of DMSO and 0.9% NaCl, 22% protein), and maintained at 23 ± 1 °C under a 12:12-h light-dark cycle. All experimental procedures were in strict accordance with our institutional guidelines and with international standards for manipulation and care of laboratory animals, being previously approved by the local Research Ethics Committee.

Table 1. Renal and systemic functional parameters before treatment (30 days after renal mass reduction) and at the end of the study (120 days after renal mass reduction)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW (120 days), g</th>
<th>LKW (120 days), g</th>
<th>TCP, mmHg</th>
<th>UalbV, mg/day</th>
<th>Screat, (120 days), mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 days</td>
<td>120 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>12</td>
<td>359±6</td>
<td>1.6±0.1</td>
<td>119±3</td>
<td>112±3</td>
<td>2.0±0.2</td>
</tr>
<tr>
<td>Nxpre</td>
<td>12</td>
<td>253±3*a</td>
<td>1.2±0.1*a</td>
<td>172±3*a</td>
<td>ND</td>
<td>104±7.1*b</td>
</tr>
<tr>
<td>Nx</td>
<td>12</td>
<td>273±9*a</td>
<td>1.2±0.1*a</td>
<td>184±5*a</td>
<td>216±6*b</td>
<td>97.4±16.4*a</td>
</tr>
<tr>
<td>NxLos</td>
<td>14</td>
<td>279±12*a</td>
<td>1.2±0.1*a</td>
<td>178±4*a</td>
<td>174±9*</td>
<td>92.6±7.5*a</td>
</tr>
<tr>
<td>NxNOF</td>
<td>14</td>
<td>270±12*a</td>
<td>1.2±0.1*a</td>
<td>175±8</td>
<td>189±4*c</td>
<td>96.2±12.8*a</td>
</tr>
<tr>
<td>NxLos/NOF</td>
<td>13</td>
<td>291±6*b</td>
<td>1.3±0.1*a</td>
<td>177±7</td>
<td>144±7*b</td>
<td>97.6±9.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats; BW, body weight; LKW, left kidney weight; TCP, tail-cuff pressure; UalbV, urinary albumin excretion; Screat, serum creatinine concentration; ND, not determined; S, sham-operated; Nx, 5/6 nephrectomy; Nxpre, 30 days after Nx; Nx, NxLos, NxNOF, and NxLos/NOF. Nx rats treated with vehicle, losartan, nitrofuribuprofen (NOF), and combination therapy, respectively. *P < 0.05 vs. S. **P < 0.05 vs. Nxpre. ***P < 0.05 vs. Nx. ****P < 0.05 vs. NxLos. P < 0.05 vs. NxLos/NOF. ∞P < 0.05 vs. NxNOF. ©P < 0.05 vs. NxLos.
enhance antigen retrieval and preincubated with 5% normal rabbit (for ED-1) or horse (for COX-2, AT1, and ANG II) serum in Tris-buffered saline to prevent nonspecific protein binding. Incubation with the primary antibody was always carried out overnight at 4°C in a humidified chamber. Negative control experiments for all antigens were performed by omitting incubation with the primary antibody.

For macrophage detection, a monoclonal mouse anti-rat ED-1 antibody (Serotec, Oxford, UK) was used. After being washed, sections were incubated with rabbit anti-mouse IgG (Dako, Glostrup, Denmark), then with an alkaline phosphatase anti-alkaline phosphatase (Dako) complex. Finally, sections were developed with a fast-red dye solution, counterstained with Mayer’s hemalum, and covered with Kaiser’s glycerin-gelatin (Merck, Darmstadt, Germany).

COX-2-, ANG II-, and AT1R-positive cells were detected by an indirect streptavidin-biotin alkaline phosphatase technique. The primary (monoclonal mouse anti-rat) antibody for COX-2 was purchased from Transduction Laboratories (Lexington, KY). For ANG II detection, a monoclonal rabbit anti-human ANG II (Peninsula Lab, San Carlos, CA) was used, whereas AT1R was detected with a monoclonal rabbit anti-rat AT1R antibody (RDI, Flanders, NJ). Sections were preincubated with avidin and biotin solutions to block nonspecific binding of these compounds (Blocking Kit, Vector Labs, Burlingame, CA). After being washed, the sections were incubated at room temperature with rat-adsorbed biotinylated anti-mouse or anti-rabbit IgG (Vector Labs) for 45 min, then with a streptavidin-biotin-alkaline phosphatase complex (Dako) for an additional 30 min. Sections were finally incubated with a freshly prepared substrate, consisting of naphthol AS-MX-phosphate and developed as described above.

The extent of renal infiltration by macrophages, ANG II-positive cells, and COX-2-positive cells was evaluated in a blinded manner at ×250 magnification and expressed as cells per square millimeter. For each section, 25 microscopic fields, each corresponding to an area of 0.06 mm², were examined. Because interstitial AT1R in Nx rats was often so densely expressed as to preclude the individualization of positively stained cells, AT1R expression had to be estimated by a point-counting technique similar to the one employed to determine %INT. This technique allowed us to assess the distribution of AT1R among several compartments of the renal cortex (glomeruli, vessels, tubules, and interstitium). The glomerular expression of COX-2 was evaluated by counting positively stained cells in a total of 100 glomeruli/rat, and the results were expressed as cells/100 glomeruli. The fraction of stained macula densa regions was also estimated. The expression of COX-2 in arteries/arterioles was evaluated by counting the number of stained cells in a total of 50 cortical vessels and given as cells/50 vascular profiles.

**Statistical analysis.** One-way ANOVA with paired comparisons according to the Newman-Keuls formulation was used in this study (9). The Spearman correlation coefficient was used to evaluate the existence of significant linear correlation between parameters obtained in individual rats. Because GSI and albumin excretion rates behaved as continuous variables with nonnormal distribution, an approximately Gaussian distribution was obtained in all groups by performing log transformation of these data before statistical analysis. For similar reasons, parameters expressed as proportions underwent arcsine transformation before analysis (9). P values < 0.05 were considered significant.

**RESULTS**

Renal and systemic parameters obtained at 30 (before treatment) and 120 days after Nx are given in Table 1. Nx groups exhibited limited growth compared with S. In all Nx groups except NxLos/NOF, body weights were not statistically different from those observed before treatment. Average food intake was similar among groups. The left kidney weight was similar among Nx groups 120 days after Nx. Figure 1 shows, in a graphic manner, TCP as a function of time. TCP rose markedly in the NxV group, reaching 216 ± 6 mmHg 120 days after surgery (vs. 112 ± 3 in S, P < 0.05, and 172 ± 3 mmHg in Nxpre, P < 0.05). After 30 days of treatment (60 days after Nx), TCP was unchanged by NOF monotherapy but fell by ~40 mmHg in both NxLos and NxLos/NOF. Los monotherapy lost part of this initial antihypertensive effect with time, TCP returning to pretreatment levels 120 days after renal ablation. Because NOF monotherapy had no antihypertensive effect, TCP reached similar values in NxLos and NxLos/NOF rats 120 days after Nx. Although these values were significantly lower than in NxV, TCP did not differ statistically from the respective Nxpre values, remaining markedly elevated compared with S. In the NxLos/NOF group, the initial antihypertensive effect was maintained throughout the study (Fig. 1). As a consequence, final TCP values in this group were only moderately elevated compared with S (144 ± 7 mmHg, P < 0.05 vs. S and NxV) and significantly lower than those verified before treatment (P < 0.05 vs. Nxpre).

As expected, UαV (Table 1) was markedly increased at 30 days of surgery (Nxpre group), reaching 104.3 ± 7.1 mg/day (P < 0.05 vs. S). Albuminuria was aggravated at 120 days after Nx (178.5 ± 43.1 mg/day, P < 0.05 vs. S and P > 0.05 vs. Nxpre). Monotherapy with LOS numerically decreased UαV relative to untreated Nx (112.9 ± 14.7 mg/day, P > 0.05 vs. NxV) and prevented further increases in albuminuria, which remained at levels similar to those observed in the Nxpre group. Monotherapy with NOF significantly reduced albuminuria relative to pretreatment values (72.6 ± 11.2 mg/day, P < 0.05 vs. NxV, NxLos, and Nxpre). Combined Los+NOF treatment exerted a much more efficient antiproteinuric action than any of the monotherapies, reversing albuminuria to 26.2 ± 3.5 mg/day (P < 0.05 vs. NxV, NxLos, NNXos, and Nxpre).

As expected, Screat levels were markedly increased by renal mass reduction. None of the monotherapies promoted a significant decline in Screat relative to either NxV or Nxpre. In the Los+NOF group, Screat was significantly reduced compared with either NxV or Nxpre (1.0 ± 0.1 vs. 1.2 ± 0.1 in Nxpre, P < 0.05 and 1.4 ± 0.2 mg/day in NxV, P < 0.05).

As shown in Table 2, urinary excretion of TxB2, measured at 30 days of treatment, was slightly increased in NxV compared
with S (20.6 ± 1.7 vs. 17.8 ± 1.9 ng/day in S, P > 0.05). Calculated TxB2 excretion per nephron was increased sevenfold in Nx rats 60 days after surgery (2.1 ± 0.2 vs. 0.3 ± 0.1 pg-nephron⁻¹-day⁻¹ in S, P < 0.05). As expected, total urinary TxB2 excretion was markedly depressed compared with S and NxV in NxNOF and NxLos/NOF rats (7.0 ± 1.0 and 8.6 ± 0.8 ng/day, respectively, P < 0.05 vs. S and NxV). TxB2 excretion in the NxNOF and NxLos/NOF groups appeared even more depressed when factored by the estimated number of nephrons, reaching values close to those verified in S (0.7 ± 0.1 and 0.9 ± 0.1 ng-nephron⁻¹-day⁻¹, respectively, P < 0.05 vs. NxV and P > 0.05 vs. S). Monotherapy with Los resulted in a slight but statistically significant reduction in the calculated TxB2 excretion per nephron relative to Nx (1.7 ± 0.2 ng-nephron⁻¹-day⁻¹, P < 0.05 vs. NxV).

Glomerular segmental sclerotic lesions were evident 30 days after surgery (Nxpre group), the GSI reaching values almost 20-fold higher than in S (Table 3). Ninety days later (120 days after surgery), considerable progression of glomerular injury had occurred. In NxV rats, the GSI attained values almost 10-fold as high as in Nxpre and 200-fold higher than in S. Treatment with any of the monotherapies was associated with a less pronounced increment of the GSI, although the respective differences relative to the NxV group were not statistically significant. Combined Los/NOF treatment arrested the progression of glomerular injury, keeping the GSI at levels close to those verified in the Nxpre group (43 ± 11 vs. 19 ± 4 in Nxpre, P > 0.05). There was a significant correlation between GSI and %INT (r = 0.74, P < 0.01). Interstitial expansion was also a prominent component of renal injury after Nx (Table 3), %INT reaching values more than threefold higher than in Nxpre 120 days after Nx. Unlike NOF and Los monotherapies, combined Los/NOF treatment significantly attenuated the progression of interstitial expansion (4.8 ± 0.5 vs. 7.8 ± 0.7 in NxV, P < 0.05).

**Immunohistochemistry.** Thirty days after Nx (Nxpre group), macrophage infiltration in the renal tissue (Table 3), assessed by the density of ED-1-positive cells, more than quadrupled compared with S values (131 ± 12 vs. 29 ± 3 cells/mm² in S, P < 0.05). This was aggravated 120 days after surgery, macrophage density reaching values of 178 ± 23 cells/mm² in the NxV group. None of the monotherapies had any significant influence on macrophage infiltration. However, the combined Los/NOF treatment reduced the renal macrophage density to levels significantly lower than in the Nxpre group (81 ± 6 cells/mm² vs. Nxpre, P < 0.05). A significant linear correlation (r = 0.67, P < 0.001) was observed between the macrophage density and the GSI.

Figure 2A shows a typical staining pattern for ANG II in an afferent arteriole obtained from an S rat (the efferent arteriole, not stained, is shown as well). In Fig. 2B, ANG II-positive cells unrelated to vascular tissue are shown in the renal cortical interstitium of an Nx rat 120 days after ablation. Most of these ANG II-positive cells appeared in association with inflamed areas. Figure 3 shows the intensity of both modalities of ANG II expression in graphic form. Afferent arteriolar ANG II expression was deeply depressed in untreated Nx (0.04 ± 0.02 positively stained arterioles/mm² in Nxpre and 0.03 ± 0.02 in NxV vs. 1.55 ± 0.16 in S, P < 0.05) and was not influenced by any of the treatments. By contrast, the density of ANG II-positive cells in the interstitium was already markedly increased at 30 days after ablation (Nxpre group) compared with S. These values were tripled 120 days after Nx (NxV group). The increasing interstitial ANG II expression was completely arrested and kept at pretreatment levels by the combined Los/NOF treatment. None of the monotherapies had any significant effect on interstitial ANG II expression.

AT1R expression patterns are shown in Figs. 4 and 5. In the S group, AT1R expression was almost entirely confined to the tubular compartment and only sparsely expressed at glomeruli, vessels, and interstitium. Renal mass reduction increased only numerically the total renal expression of AT1R but promoted a drastic change in its intrarenal distribution. In the Nxpre group (30 days after Nx), total renal AT1R expression was clearly shifted to the interstitial area. This pattern became even more marked 120 days after ablation (NxV group), when AT1R expression at the tubules declined to very low levels (Fig. 5). None of the treatments promoted any significant change in the intensity or distribution of AT1R expression in Nx rats.

As described previously (9, 49), COX-2 was constitutively expressed in cells of the macula densa region of S rats (Fig. 6A). Only rare COX-2-positive cells were found in glomeruli, vessels, or interstitium of intact kidneys. In accordance with previous observations of this laboratory (9), renal mass reduction numerically increased the frequency of macula densa staining positively for COX-2 (Table 4). Monotherapy with NOF or Los further augmented the expression of COX-2 at the macula densa, although only with the latter was this change statistically significant (Table 4). Combined Los/NOF treatment nearly doubled COX-2 expression at the macula densa (Table 4).

As previously reported by this laboratory (9), there was a dramatic elevation in the density of COX-2-positive cells at glomeruli (Fig. 6B), vessels (Fig. 6C), and interstitium (Fig.

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**Table 2. Urinary thromboxane B2 excretion 60 days after renal ablation (30 days of treatment)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total TxB2, Excretion, ng/day</th>
<th>Estimated per Nephron TxB2 Excretion, pg-nephron⁻¹-day⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>17.8 ± 1.9</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>NxV</td>
<td>20.6 ± 1.7</td>
<td>2.1 ± 0.2*</td>
</tr>
<tr>
<td>NNXls</td>
<td>16.5 ± 2.4</td>
<td>1.7 ± 0.2*</td>
</tr>
<tr>
<td>NNXNOF</td>
<td>7.0 ± 1.0*</td>
<td>0.7 ± 0.1*</td>
</tr>
<tr>
<td>NNXLos/NOF</td>
<td>8.6 ± 0.8*</td>
<td>0.9 ± 0.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. TxB2, thromboxane B2.*P < 0.05 vs. S. †P < 0.05 vs. NxV.

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**Table 3. Parameters of renal injury and inflammation 120 days after renal ablation (90 days of treatment)**

<table>
<thead>
<tr>
<th>Group</th>
<th>GSI</th>
<th>%INT</th>
<th>Mn (cells/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>1 ± 1</td>
<td>0.6 ± 0.1</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>Nxpre</td>
<td>19 ± 4a</td>
<td>2.5 ± 0.3a</td>
<td>131 ± 12a</td>
</tr>
<tr>
<td>NxV</td>
<td>189 ± 38ab</td>
<td>7.8 ± 0.7ab</td>
<td>178 ± 23a</td>
</tr>
<tr>
<td>NxLos</td>
<td>134 ± 31ab</td>
<td>6.8 ± 1.0ab</td>
<td>155 ± 19ab</td>
</tr>
<tr>
<td>NxNOS</td>
<td>130 ± 37ab</td>
<td>7.9 ± 1.2ab</td>
<td>140 ± 13ab</td>
</tr>
<tr>
<td>NNXlos/NOF</td>
<td>43 ± 11abde, f</td>
<td>4.8 ± 0.5abcdef, f</td>
<td>81 ± 6abcdef, f</td>
</tr>
</tbody>
</table>

Values are means ± SE. GSI, glomerular sclerosis index; %INT, fractional cortical interstitial area; Mn, extent of macrophage infiltration. *P < 0.05 vs. S; †P < 0.05 vs. Nxpre; ‡P < 0.05 vs. NxV; ′P < 0.05 vs. NNXlos. ′′P < 0.05 vs. NNXNOF.
As expected, % renal ablation promoted growth retardation, systemic arterial hypertension, impaired renal function, and severe albuminuria. These functional changes were accompanied by severe glomerulosclerosis, as well as expansion and cellular infiltration of the interstitial area. Mounting evidence indicates that these renal structural abnormalities, which are characteristic of the Nx and other models of progressive nephropathies, are a consequence of the concerted action of mechanical stress, caused by glomerular hypertension and hypertrophy (2, 9), and inflammatory phenomena, comprising cell infiltration and/or proliferation and extracellular matrix accumulation (8, 9). Moreover, a causal relationship appears to exist between these phenomena, because the distension of the glomerular walls due to intracapillary hypertension may trigger the local release of cytokines, growth factors, and, particularly, ANG II and prostanooids (1, 23).

As described previously, intrarenal ANG II distribution was profoundly changed after renal mass reduction (28). Thirty days after ablation there was a marked decrease in ANG II expression in afferent arterioles, whereas large amounts of ANG II-positive cells appeared in the inflamed interstitial areas. This intrarenal shift of ANG II-positive cells was intensified 120 days after Nx, which may have contributed substantially to aggravate renal injury. These findings support and extend previous observations made in Nx and chronic nitric oxide (NO) inhibition models, which showed the presence of ANG II-positive cells in inflamed areas of the renal interstitium (28, 34). The pattern of intrarenal ANG II distribution suggests completely distinct roles for ANG II located in each of these “compartments.” It is possible that “vascular” ANG II, the expression of which is reduced after renal ablation, is primarily linked to regulation of sodium balance. According to this view, vascular ANG II would be depressed by the extracellular fluid volume expansion known to occur in the Nx model (17). By contrast, “interstitial” ANG II seems to be insensitive to volume expansion and, given its association with interstitial expansion and cellular infiltration, it appears to be linked to renal inflammation. The origin of this interstitial ANG II was not addressed in the present study. ANG II may have been produced locally, because tubular epithelial cells, macrophages, and myofibroblasts all possess the appropriate biochemical machinery (12, 29, 39). Alternatively, ANG II may have originated outside the renal interstitium and undergone internalization by local cells after binding to AT1R (44, 57). Local ANG II may have had a proinflammatory and profibrotic effect at the renal interstitium. ANG II stimulates inflammatory cells such as lymphocytes (25) and activates nuclear factor-κB in monocytes (36). In addition, ANG II stimulates the release of MCP-1 by vascular smooth muscle cells (36). In renal tissue, ANG II stimulates the proliferation of mesangial cells, glomerular endothelial cells (53), and myofibroblasts (55), as well as the secretion of chemokines and growth factors such as RANTES (52), PDGF (8), and MCP-1 (36). In addition, ANG II strongly stimulates collagen synthesis and renal fibrosis by activating TGF-β (54) and the MAPK/ERK pathway (41). Blockade of interstitial ANG II is a possible explanation for the...
well-known beneficial effect of RAS suppressors in progressive nephropathies, also observed in the NxLos and NxLos/NOF groups.

In agreement with previous observations (15), renal expression of AT1R in S rats was typically expressed in tubules (T), vessels (V) and, to a much lesser extent, glomeruli (G). In Nxpre rats (30 days after ablation), AT1R expression was massively shifted to the interstitium. In NxV rats (120 days after ablation), interstitial AT1R expression was even more intense, whereas tubular and vascular AT1R expression was much weaker than in the Nxpre group.

The potential role of prostanoids in the pathogenesis of progressive nephropathies has long been acknowledged. The stimulation of podocytes by complement fractions can increase the local synthesis of prostanoids (40). Similarly, nonimmune mechanisms such as mesangial stretching can augment the expression of COX and enhance the production of its derivatives (1). Accordingly, studies of the Nx model showed increased urinary excretion of prostanoids per nephron (26).

Increased production of prostanoids can enhance inflammation and, therefore, accelerate renal injury. Prostanoids derived from COX-2 are thought to modulate proliferation and activation of T lymphocytes (20). Dendritic cells, described in the remnant kidney (33), constitutively express COX-2 and utilize prostanoids as an autocrine stimulus for cytokine secretion and for their own proliferation (51). In addition to its well-known vasoconstrictor effect (24), TXA2 stimulates the expression of adhesion molecules and of MCP-1 in endothelial cells (21), promotes the proliferation of mesangial cells (4), and enhances platelet aggregation and extracellular matrix production (5, 24).

Previous studies have shown that the renal cortical expression of COX-2 increases after 5/6 renal ablation, whereas the expression of COX-1 remains unchanged (18, 49). We showed recently that a large fraction of the excess COX-2 expressed in remnant kidneys localizes in glomeruli, vessels, and the interstitium, especially in areas of injury and inflammation (9). The present study confirms these observations, reinforcing the concept that COX-2 can exert a dual role in this model: at the MD, COX-2 appears to exert a physiological effect, possibly related to sodium homeostasis. At “anomalous” locations such as

![Graphical representation of renal AT1R distribution among tubular (dark gray bar sections), interstitial (light gray bar sections), glomerular (open bar sections), and vascular (filled bar sections) compartments. Error bars (SE) refer to total height of column (total renal AT1R). *P < 0.05 vs. S (tubular compartment).](http://ajprenal.physiology.org/)

Fig. 4. AT1 receptor (AT1R) expression (positive structures appear heavily stained). A: in S rats, AT1R was typically expressed in tubules (T), vessels (V) and, to a much lesser extent, glomeruli (G). B: in Nxpre rats (30 days after ablation), AT1R expression was massively shifted to the interstitium. C: in NxV rats (120 days after ablation), interstitial AT1R expression was even more intense, whereas tubular and vascular AT1R expression was much weaker than in the Nxpre group.
glomeruli and vessels, COX-2 and its products would mediate inflammation and structural injury. The consistent presence of COX-2 in damaged areas, and the strong correlation between the intensity of its expression and parameters of renal injury, strengthens the notion that COX-2-derived prostanoids play an important pathogenic role in this model. The mechanisms by which COX-2 may have been induced in these areas are obscure. COX-2 may have been activated by ANG II anomalously produced in the interstitium (56), by stretching of mesangial cells resulting from glomerular hypertension (1) and/or by the action of other mediators such as TNF-α and interleukin-1β (7, 31). Once synthesized, prostanoids can further activate COX-2, thereby contributing to amplify and perpetuate the inflammatory process (42). The protective effect of chronic treatment with either COX-2-specific inhibitors (9, 48) or NOF (10) lends further support to the notion that prostanoids play a fundamental role in the pathogenesis of progressive renal injury in the Nx model.

Consistent with previous observations, Los monotherapy lowered blood pressure by 40 mmHg 1 mo after treatment was started, although TCP returned to pretreatment levels at the end of the study. In addition, Los limited Ua/5V, GSI, interstitial damage, macrophage infiltration, and ANG II-positive cell infiltration (11). However, protection conferred by losartan monotherapy was only partial, because progression of renal inflammation and of renal structural injury was not arrested in the N\textsubscript{Nx,Los} group. There are at least three possible reasons for the limited efficacy of Los treatment in this study. First, rats failing to attain blood pressures higher than 140 mmHg or albuminuria in excess of 50 mg/dl 30 days after nephrectomy were excluded from the study to ensure that the attending nephropathy had a progressive nature. Second, unlike in most previous studies of this model, treatments were started only 30 days after nephrectomy, when renal injury was already established. Third, rats were followed up to 4 mo after renal mass reduction, whereas in most other studies of this model observations were ended at 2 mo or less. In the face of the unusual severity of renal injury, the relative resistance to Los treatment was not unexpected. At any instance, these findings agree with previous experimental observations, obtained in this laboratory and elsewhere (11, 19), as well as in large clinical trials (3), all of which indicate that, once set in motion, progressive nephropathies can be attenuated, but not detained, by isolated treatment with AT\textsubscript{1}R blockers or ACE inhibitors. As a whole, these observations suggest that events antecedent to the initiation of treatment may be of crucial importance in the pathogenesis of renal injury associated with this model. Additionally, the relative inefficiency of Los monotherapy in the present study may reflect the presence of ANG II-independent inflammatory events, as well as the recrudescence of arterial hypertension after the first few weeks of treatment. Finally, it is conceivable that the “conventional” dose of Los employed in the present study, although high enough to exert full vascular effect, was insufficient to effectively block the enormous

Table 4. Renal COX-2 expression and its distribution

<table>
<thead>
<tr>
<th>Group</th>
<th>Glomeruli, + cells/100 glom.</th>
<th>MD, + MD/100 glom.</th>
<th>Vascular, + cells/50 vasc. profiles</th>
<th>Intersitial, + cells/mm(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>2.0 + 0.7</td>
<td>18 + 1.5</td>
<td>1.4 + 0.8</td>
<td>0.9 + 0.2</td>
</tr>
<tr>
<td>N\textsubscript{Nx,pre}</td>
<td>30.4 + 4.1(^a)</td>
<td>24 + 1.6</td>
<td>27.9 + 10.2(^a)</td>
<td>10.4 + 2.7(^a)</td>
</tr>
<tr>
<td>N\textsubscript{Nx}</td>
<td>106.2 + 27.7(^a)</td>
<td>21.5 + 4</td>
<td>30.3 + 7.5(^a)</td>
<td>10.8 + 2.0(^a)</td>
</tr>
<tr>
<td>N\textsubscript{Nx,Los}</td>
<td>60.5 + 22.9(^a)</td>
<td>31.8 + 5</td>
<td>18.0 + 8.7(^a)</td>
<td>9.9 + 4.0(^a)</td>
</tr>
<tr>
<td>N\textsubscript{Nx,Los,NOF}</td>
<td>97.6 + 30.7(^a)</td>
<td>26.3 + 4.6</td>
<td>29.6 + 11.8(^a)</td>
<td>8.8 + 2.4(^a)</td>
</tr>
</tbody>
</table>

Values are means ± SE. MD, macula densae; + cells, cells staining positively for COX-2; + MD, COX-2-positive maculae densae. \(^aP < 0.05\) vs. S; \(^bP < 0.05\) vs. N\textsubscript{Nx,pre}; \(^cP < 0.05\) vs. N\textsubscript{Nx}; \(^dP < 0.05\) vs. N\textsubscript{Nx,Los}; \(^eP < 0.05\) vs. N\textsubscript{Nx,Los,NOF}.
amount of AT1R already present at the renal tissue when treatment was started.

NOF is a nonsteroidal anti-inflammatory compound with low gastrointestinal toxicity, presumably due to its NO-releasing properties (10, 47). It appears unlikely that NO released by NOF has a direct therapeutic effect because the NOF molecule is rapidly degraded in the intestinal lumen, releasing flurbiprofen (10). However, we cannot exclude the possibility that nitroso proteins, which have a much longer half-life than NO itself, propagate a possible protective effect of NO released into the intestinal lumen (37). Flurbiprofen is a poten

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