ACE inhibition increases expression of the ET<sub>B</sub> receptor in kidneys of mice with unilateral obstruction

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Moridaira, Kazuaki, Jeremiah Morrissey, Melanie Fitzgerald, Guangjie Guo, Ruth McCracken, Timothy Tolley, and Saulo Klahr. ACE inhibition increases expression of the ET<sub>B</sub> receptor in kidneys of mice with unilateral obstruction. *Am J Physiol Renal Physiol* 284: F209–F217, 2003; 10.1152/ajprenal.00352.2001.—Unilateral ureteral obstruction (UUO) is a well-established model for the study of interstitial fibrosis in the kidney. It has been shown that the renin-angiotensin system plays a central role in the progression of interstitial fibrosis. Recent studies indicate that endothelin, a powerful vasoconstrictive peptide, may play an important role in some types of renal disease. To investigate the effects of angiotensin II on endothelin and its receptors in the kidney, mice were subjected to UUO and treated with or without enalapril, an orally active angiotensin-converting enzyme inhibitor, in their drinking water (100 mg/l). The animals were killed 5 days later. Using RT coupled with PCR, we measured the levels of endothelin-1, endothelin A, and endothelin B (ET<sub>B</sub>) along with transforming growth factor-β, TNF-α, and collagen type IV mRNA expression in the kidney with UUO and the contralateral kidney along with interstitial expansion in the kidney cortex by a standard point counting method. We found that enalapril administration ameliorated the increased expression of ET-1 mRNA in the obstructed kidney by 44% (∗P < 0.02). Although the level of endothelin A mRNA expression was significantly increased in the obstructed kidney, it was not affected by enalapril. We found that enalapril treatment increased ET<sub>B</sub> mRNA expression by 115% (∗P < 0.05) and protein expression (measured by Western blot) in the kidney with an obstructed ureter. Enalapril treatment alone inhibited the expansion of interstitial volume due to UUO by 52%. Cotreatment with enalapril and the ET<sub>B</sub> receptor antagonist BQ-788 inhibited the expression of interstitial volume by only 19%. This study confirms that enalapril inhibits the interstitial fibrosis in UUO kidneys. It also suggests a beneficial and unforeseen effect of enalapril on the obstructed kidney by potentially stimulating the production of nitric oxide through an increased expression of the ET<sub>B</sub> receptor.

nitric oxide formation; fibrosis; enalapril; angiotensin-converting enzyme; endothelin B receptor

TUBULOINTERSTITIAL FIBROSIS develops in a variety of kidney diseases (29, 30, 33). Available data indicate that angiotensin II plays a central role in the initiation and progression of renal disease by autocrine, paracrine, interocrine, and endocrine pathways. Experimental ureteral obstruction is a well-established model for the study of interstitial fibrosis (20, 25, 26). Intrarenal concentrations of angiotensin II increase rapidly after the onset of ureteral obstruction in the ligated obstructed kidney (10). Angiotensin II, in turn, upregulates the expression of transforming growth factor-β (TGF-β), TNF-α, and other growth factors and cytokines that lead to the accumulation of ECM proteins and to renal damage (20–23, 28). We previously reported the beneficial effects of angiotensin-converting enzyme (ACE) inhibitor on the progression of tubulointerstitial fibrosis in the UUO model (19–21, 23, 28). ACE inhibitors were found to blunt TGF-β and TNF-α expression concomitant with amelioration of histological changes that occur in the kidney during disease. A more precise identification of these other factors that contribute to the initiation of and/or progression of kidney fibrosis is the subject of the study.

In the last decade, a number of studies have suggested that endothelin, a powerful vasoconstrictive peptide (48), is also involved in the progression of chronic renal disease (2, 15, 31). Endothelin has at least three isopeptides: endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3) (18). Their effects are mediated through two different receptors: endothelin A (ET<sub>A</sub>), selective for ET-1 and ET-2, and endothelin B (ET<sub>B</sub>), nonselective to the three ET isopeptides (16, 46, 47). Among the three ETs, ET-1 appears to be the most important in pathophysiological conditions in the kidney. Indeed, it has been shown that renal tissue can synthesize and release ET-1 and also expresses both ET<sub>A</sub> and ET<sub>B</sub> receptors (3).

It has been shown in the last few years that angiotensin II can stimulate the synthesis and release of ET-1 in endothelial cells or vascular smooth muscle cells (7, 9, 12, 34, 44). However, the role and interactions between angiotensin II and ET-1 in the progression of renal fibrosis due to ureteral obstruction are still unclear. Therefore, we used the ACE inhibitor...
enalapril to explore the effects of angiotensin II on the mRNA expression of ET-1, ET<sub>A</sub>, and ET<sub>B</sub> in mice with UUO. RT-PCR was utilized for the semiquantitative analysis of mRNA. To confirm the effects of enalapril, we also measured other cytokines, such as TGF-β, TNF-α, and collagen type IV mRNA, as a marker of ECM proteins and evaluated histological changes by using a standard point counting method. This would validate any observed change(s) in the pattern of endothelin gene expression. This would help to integrate the contribution of the endothelin in the progression of renal disease that probably was initiated by the increase in angiotensin II.

**MATERIALS AND METHODS**

**Animals and reagents.** Female C57BL/6 mice (~25–30 g) were purchased from Harlan (Indianapolis, IN). Enalapril, BQ-788, and Tri Reagent, a reagent for RNA isolation, were supplied by Sigma (St. Louis, MO). Avian myeloblastosis virus reverse transcription kits and Taq polymerase were obtained from Promega (Madison, WI).

**Experimental design.** Initially, mice were divided into two groups: those receiving enalapril in the drinking water (100 mg/l; n = 9) and those receiving water alone as a control (n = 9). Each group was given water with or without enalapril from 1 day before obstruction of the ureter through 5 days of obstruction. Unilateral ureteral obstruction (UUO) was performed as described previously (19-23). In brief, a midline abdominal incision was made while the mice were under anesthesia, and both ureters were exposed. The left ureter was ligated with 4–0 silk at one-third the distance from the bladder to the kidney. Animals were allowed to drink or eat normal rodent chow ad libitum after surgery. Subsequently, additional groups of mice were prepared to receive enalapril in the drinking water with or without daily injections (ip) of the ET<sub>B</sub> receptor antagonist BQ-788 at a dose of 1 mg/kg (48).

After 5 days of UUO, the mice were anesthetized with an overdose of ketamine HCl-xylazine HCl, and the kidneys were immediately harvested, decapsulated, and washed in ice-cold PBS. The kidneys were prepared for total RNA isolation and histological examination as described previously (19-23).

**Preparation of RNA.** Total RNA was isolated from each kidney by the guanidinium-thiocyanate method (5). In brief, portions of the kidneys were homogenized in 1 ml of Tri Reagent. Total RNA was precipitated with isopropanol. The RNA pellets were washed in 75% ethanol, air dried, and dissolved in RNase-free distilled water. The quantitative analysis of total RNA was performed at 260 and 280 nm.

**RT-PCR.** Total RNA extracted from the kidneys was reverse transcribed into first-strand cDNA in RT buffer (10 mM Tris-HCl, 50 mM KCl, 5 mM MgCl2, 1 mM deoxynucleotide triphosphate mixture, and 1 U/μl RNase inhibitor), 0.5 U/μl avian myeloblastosis virus RT, and oligo(dT) primers. The incubation conditions (42°C for 1 h followed by 95°C for 5 min) were established by using a DNA Thermal Cycler (Perkin-Elmer).

**PCR amplification** was performed for ET-1, ET<sub>A</sub>, ET<sub>B</sub>, TGF-β, TNF-α, collagen type IV, and GAPDH with the primers shown in Table 1. Successively, each of the cDNAs were amplified in PCR amplification buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl2, and 200 μM deoxynucleotide triphosphate mixture) containing 1.25 U/μl Promega AmpliTaq DNA polymerase and 25 pM primers for each amplification under the following conditions: denaturing at 94°C for 1 min, annealing at different temperatures (Table 1) for 1 min, and extension at 72°C for 1 min up to their optimal cycles (Table 1). Preliminary studies were performed to determine the appropriate number of cycles needed for linear amplification of the target DNA. In addition, each pair of primers was confirmed to amplify the objective cDNA by digestion of the product with at least two restriction enzymes and dideoxy chain-termination DNA sequencing.

**Relative quantitative analysis of mRNA.** Products amplified by PCR were separated by 2% agarose gel containing ethidium bromide. The gels were visualized with UV light and were photographed with Polaroid Type 665 positive-negative films. The intensity of bands was measured by densitometry for quantification. The relative level of each mRNA was determined by normalizing the quantity of specific cDNA to the amount of GAPDH cDNA.

**Relative quantitative analysis of ET<sub>B</sub> protein.** Portions of kidney cortex were solubilized with Laemmli sample buffer by heating to 95°C for 5 min followed by brief centrifugation after cooling. The protein content of the supernatant was determined by Bradford assay and diluted to 2 mg/ml of sample. Twenty-five microliters of total protein were separated by means of 10% acrylamide gels containing sodium dodecyl sulfate. Gel lanes containing kidney extracts were flanked by lanes containing prestained molecular mass

**Table 1. Primers for polymerase chain reaction**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Annealing Temperature, °C</th>
<th>Cycles</th>
<th>Product Size, bp</th>
<th>Reference no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>Sense</td>
<td>5'-AAATGATCATGTCGACCCAAA</td>
<td>58</td>
<td>515</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5'-CATGCTATTTCTGAAAAGAAA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β1</td>
<td>Sense</td>
<td>5'-AAATGATCATGTCGACCCAAA</td>
<td>60</td>
<td>28</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5'-CATGCTATTTCTGAAAAGAAA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>Sense</td>
<td>5'-AAATGATCATGTCGACCCAAA</td>
<td>60</td>
<td>28</td>
<td>38</td>
</tr>
<tr>
<td>Collagen IV</td>
<td>Sense</td>
<td>5'-AAATGATCATGTCGACCCAAA</td>
<td>60</td>
<td>28</td>
<td>38</td>
</tr>
<tr>
<td>ET-1</td>
<td>Sense</td>
<td>5'-AAATGATCATGTCGACCCAAA</td>
<td>60</td>
<td>28</td>
<td>38</td>
</tr>
<tr>
<td>ET&lt;sub&gt;A&lt;/sub&gt;</td>
<td>Sense</td>
<td>5'-AAATGATCATGTCGACCCAAA</td>
<td>60</td>
<td>28</td>
<td>38</td>
</tr>
<tr>
<td>ET&lt;sub&gt;B&lt;/sub&gt;</td>
<td>Sense</td>
<td>5'-AAATGATCATGTCGACCCAAA</td>
<td>60</td>
<td>28</td>
<td>38</td>
</tr>
</tbody>
</table>

TGF-β1, transforming growth factor-β1; ET-1, endothelin-1; ET<sub>A</sub> and ET<sub>B</sub>, endothelin A and B, respectively.
markers. Proteins in the acrylamide gel were electrophoretically transferred to nitrocellulose membranes in Tris-glycine buffer containing 20% methanol with the prestained markers used as a guide for transfer efficiency. The location and relative amount of ET<sub>B</sub> antibody (1:100) were prepared in rabbit catalog no. AER-002 (Alomone Labs, Jerusalem, Israel) followed by a goat anti-rabbit IgG-horseradish peroxidase conjugate (Santa Cruz Biotechnology, Santa Cruz, CA) and Luminol reagent SC-2048. Similar results were obtained with an anti-rat ET<sub>B</sub> antibody 1:100 made in sheep (no. 324755, Calbiochem-Nova Biochem, San Diego, CA) and a donkey anti-sheep IgG-horseradish peroxidase conjugate (A3415, Sigma).

**Immunohistochemical analysis.** Coronals sections of kidney were placed in the fixative Histochoke (Amresco, Solon, OH). The sections were dehydrated, embedded in paraffin, cut into 4-μm sections, dewaxed, and rehydrated as described previously (41). The rehydrated sections were treated overnight at 4°C with 1:100 dilutions of rabbit anti-rat ET<sub>B</sub> antibody (Alomone) followed by an alkaline phosphatase conjugated goat anti-rabbit IgG (A9919, Sigma) and evaluated with Sigma FAST-Fast Red/Naphthol (F4648).

**Morphometric analysis.** A standard point counting method was used to quantitate the volume of the renal interstitium (39). The relative volume of the renal cortical interstitium (V<sub>c</sub>/V<sub>i</sub>) was determined on sections by using Mason-Trichrome stain. Ten separate nonoverlapping microscopic fields of each kidney section were averaged to yield the score of each kidney. Each microscopic field contained one glomerular cross section to maintain consistency. The score for 9–11 separate animals for each treatment modality was then averaged.

**Statistical analysis.** All data represent means ± SD. Intergroup comparisons were performed by ANOVA and Student’s t-test. P < 0.05 was taken as the criterion of significance.

**RESULTS**

**RT-PCR.** We measured the expression of TGF-β, TNF-α, and collagen IV mRNA in mice with UUO with or without enalapril in the drinking water. This served as a measure of the effectiveness of the enalapril treatment to gauge any subsequent effects on endothelin gene family members.

Figure 1 shows the mRNA expression of TGF-β in UUO mice treated with or without enalapril. TGF-β mRNA was significantly increased in the obstructed kidney compared with the contralateral kidney (P < 0.01) of mice without enalapril. Enalapril treatment decreased the expression of TGF-β in the obstructed kidney by 74% (P < 0.05), whereas it had no effect on the contralateral kidney.

There was also a significant increase of TNF-α mRNA in the obstructed kidney (P < 0.01) compared with the contralateral kidney in untreated animals. Enalapril blunted the increased expression of TNF-α mRNA in the obstructed kidney (28%), which was statistically significant (P < 0.001). Enalapril had no effect on the expression of TNF-α mRNA in the contralateral kidney (Fig. 2).

The expression of collagen IV mRNA in the obstructed kidney was threefold greater than in the contralateral kidney (P < 0.01). This increment in the obstructed kidney was reduced (39%) by enalapril treatment. This was statistically significant (P < 0.003). Enalapril had no effect on the collagen type IV mRNA content of the contralateral kidney (Fig. 3).

**ET-1 mRNA expression was fivefold greater in the obstructed kidney than in the contralateral kidney (P < 0.001) from untreated mice. Enalapril decreased the expression of ET-1 mRNA in the obstructed kidney by 44% (P < 0.02). Enalapril did not significantly affect the expression of ET-mRNA in the contralateral kidneys (Fig. 4).

**ET<sub>A</sub>** mRNA in the obstructed kidney was 70–85% higher than that of the contralateral kidney (P < 0.05). Enalapril had no statistically significant effect on the expression of ET<sub>A</sub> mRNA in the obstructed or the contralateral kidney (Fig. 5).

No significant changes were observed in the expression of ET<sub>B</sub> mRNA between the obstructed and contralateral kidneys of animals receiving water. Enalapril treatment increased the expression of ET<sub>B</sub> mRNA in the obstructed kidney by 115% (P < 0.02). Enalapril treatment had no effect on ET<sub>B</sub> mRNA in the contralateral kidney (Fig. 6), although there was a 49% increase due to the presence of ACE inhibition. Another group of animals (n = 3 each) was subjected to a sham operation in which the ureter was manipulated but not ligated or to UUO for 5 days. The ET<sub>B</sub> mRNA content was not significantly different (0.20 ± 0.13, 0.24 ± 0.06, and 0.26 ± 0.10) for the kidneys of sham-operated mice, the
contralateral kidneys, or the kidneys with an obstructed ureter, respectively. At the same time, the amount of ET-1 mRNA was significantly increased greater than threefold in the kidney with an obstructed ureter but was not different in the contralateral kidneys from that of the kidneys from sham-operated mice.

Western blot analysis of total protein extracts from the kidneys with an obstructed ureter revealed a threefold increase ($^*P < 0.001$ vs. CK, $^{**}P < 0.02$ vs. OBK) in the amount of ET B protein at $53 \text{ kDa}$ between untreated animals ($0.37 \pm 0.10$ relative densitometer units) and enalapril-treated animals ($1.12 \pm 0.10$ relative densitometer units) (Fig. 7). This band at $53 \text{ kDa}$ is consistent with previous reports of the approximate size of the ET B receptor (31). This increase in ET B protein appears to be uniform throughout tubules of the renal cortex (Fig. 7). Only a few cells of glomeruli appear to contain ETB.

Morphometric analysis. The relative volume of the cortical interstitium was expressed as $V_{\text{vint}}$ (Fig. 8). UUO of 5 days duration showed a significant increase ($P < 0.001$, $n = 9$) of $V_{\text{vint}}$ in the obstructed kidney ($37.0 \pm 1.1\%$) compared with the contralateral kidney ($6.0 \pm 0.1\%$). This is consistent with our previous studies (23, 41) and reflects what is observed in human ureteral obstruction (39). Enalapril treatment signifi-
cantly ameliorated ($P < 0.001$) the increment by 52% in the kidneys with an obstructed ureter ($23.7 \pm 1.4\%$, $n = 11$). No significant difference was observed between the contralateral kidneys of untreated mice and mice treated with enalapril. Other mice ($n = 10$) were treated with a combination of oral enalapril and an intraperitoneal injection of the ET B-specific receptor antagonist BQ-788 (48). The decrease in interstitial volume due to enalapril treatment was significantly ($P < 0.001$) blunted by the ET B antagonism (Fig. 9). There remained a significant decrease ($P < 0.002$) between enalapril-BQ-788 cotreatment and untreated mice with UUO with respect to the change in $V_{\text{int}}$.

**DISCUSSION**

In this study, we found that enalapril, an ACE inhibitor, blunted the increased expression of ET-1 mRNA by 44% ($P < 0.02$) in the obstructed kidney. In the setting of UUO, the renin-angiotensin system is upregulated (10). Angiotensin II, in turn, stimulates the expression of TGF-β, TNF-α, collagen IV, and various cytokines or vasoactive compounds that play roles in the progression of tubulointerstitial fibrosis (20–23, 28). We have previously shown that enalapril administration blunted the increased expression of TGF-β and collagen IV mRNA in UUO rats (19–21, 23). In this study, enalapril significantly suppressed the increase of TGF-β mRNA in the kidney of mice with an obstructed ureter. These data suggest that angiotensin II either directly or indirectly upregulates ET-1 expression in the kidney with an obstructed ureter. However, the fact that ET-1 mRNA expression in the kidney with an obstructed ureter did not return to the normal level because of ACE inhibition indicates the existence of
other factor(s) involved in the upregulation of ET-1 in the obstructed kidney.

Recently, Feldman et al. (11) reported on the levels of ETA and ETB mRNA expression in UUO rats at 5 days after the onset of obstruction. They showed that ETA mRNA expression was significantly elevated in the obstructed kidney compared with the contralateral kidney. The level of ETB mRNA expression was not affected by ureteral ligation. We obtained similar results in UUO mice with ureteral obstruction of the same duration. It seems there is not a difference between rats and mice concerning the expression or change of ETA and ETB mRNA in the model of UUO.

Enalapril administration had no effect on the expression of ETA mRNA in both kidneys when compared with those not receiving enalapril. Unexpectedly, enalapril administration increased the level of ETB mRNA expression by 115% (P < 0.02) and the amount of protein threefold (P < 0.001) in the obstructed kidney. This suggests the existence of a feedback system between ET-1 and its receptor ETB. Lehrke et al. (35) found that ACE inhibition decreases ETB expression in biopsy specimens obtained from patients with chronic renal disease. Our study is measuring more acute effects.

In addition to lowering angiotensin levels, ACE inhibitors affect kininase II and increase the levels of bradykinin (10), which in turn modulate nitric oxide production by the endothelial cells (36, 40, 52). Nitric oxide may have two opposite effects on renal disease. It is known that a moderate amount of nitric oxide is beneficial in the prevention of experimental renal disease (41). It has been reported that nitric oxide has a direct effect on matrix protein synthesis (32, 51). Our laboratory has previously shown an effect of nitric oxide on the prevention of interstitial fibrosis caused by UUO by the administration of L-arginine, a nitric oxide donor (41). On the basis of those data, we suggested that one of the reasons for the beneficial effects of enalapril was the increased production of nitric oxide modulated by the upregulated bradykinin in the kidney with an obstructed ureter. On the other hand, ET-1 has various biological effects and acts quite differently depending on its binding to two different types of receptors (4, 31). ET-1 can also increase the production of nitric oxide by the endothelial cells through the ETB receptor (8). In the kidney, ETB is expressed at ~10 times the level of ETA throughout the tubule epithelium (6, 31, 49, 50). Our data suggest that enalapril may ameliorate the pathology of the obstructed kidney by increasing the level of nitric oxide not only through the kinin-bradykinin system but also by upregulation of the ETB receptor. Interestingly, Wong et al. (53) reported the downregulation of ETB in cardiomyopathic hamsters and showed that enalapril therapy...
restored ET<sub>B</sub> receptor density in these animals. In another study, renal injury was exacerbated in rats genetically deficient in the ET<sub>B</sub> receptor compared with normal rats in a doxycorticosterone-salt hypertension model (38). Furthermore, Forbes et al. (13) have shown that a dual ETA-ET<sub>B</sub> antagonist exacerbated long-term abnormalities in renal function consequent to an ischemic episode. This suggests that the ET<sub>B</sub> has an overall beneficial effect on renal pathophysiological processes. In some experimental models of renal disease, ETA expression is increased (35, 42, 55). In a previous study of a rabbit model of partial bladder outlet obstruction, there was a downregulation of ET<sub>B</sub> receptors in the medulla (24). This was measured 6 wk after partial bladder obstruction, whereas our study focuses on the renal cortex and more acute effects of obstructive nephropathy. Our study points to a beneficial effect of the ET<sub>B</sub> receptor on the expansion of interstitium as blockade with BQ-788 partially but significantly reversed the effects of enalapril. Additional studies measuring nitric oxide production and blockade of the bradykinin B2 receptor would help to fully elucidate the beneficial mechanisms of ACE inhibition.

In the present study in the kidney with an obstructed ureter, enalapril ameliorated the mRNA expression of TGF-β and TNF-α major cytokines in the regulation of ECM proteins. Morphometric analysis also showed that enalapril treatment significantly suppressed the expansion of the interstitium in the obstructed kidney. These results are consistent with our previous data in rats and mice (19–21). The dose of enalapril might need to be tailored for the model of renal disease, because Ikoma et al. (17) showed the greater effect with low dose (50 mg/l) than high dose (200 mg/l). Ikoma et al. focused on glomerular fibrosis, whereas our laboratory has focused on tubulointerstitial fibrosis (19–23).

Administration of enalapril did not decrease TNF-α mRNA levels on a percent basis in the kidney with an obstructed ureter as much as it decreased TGF-β mRNA. This is consistent with previous observations of the rats (10) and mice (29) in which enalapril treatment succeeded in blunting TNF-α mRNA expression but not to the extent seen for TGF-β. Angiotensin II appears to contribute to the early phase of increase in TNF-α mRNA expression in the obstructed kidney (10). The source of TNF-α synthesis may be different according to the time after the obstruction and level of ACE inhibition.

Again, using mice with UUO, we have shown the beneficial effects of enalapril on the progression of interstitial fibrosis and expansion due to the unilateral ureteral ligation. This is a well-established observation. Enalapril administration blunted the increased mRNA expression TGF-β and to a great degree ameliorated the histological appearance of tubulointerstitial fibrosis in the obstructed kidney of mice with UUO. However, the level of TGF-β mRNA and the histological change in the obstructed kidney did not return completely to the control level even when treated with enalapril. These results strongly suggest the existence of other factors besides angiotensin II that are involved in the progression of interstitial fibrosis in this setting or the escape of some pathophysiological factor to ACE inhibition. The present results point to endothelin expression as being a factor, in part, in renal fibrosis.

In summary, we have shown that enalapril significantly but incompletely blunts the increased expression of ET-1 mRNA in the obstructed kidney from UUO mice at 5 days after the onset of obstruction. To evaluate the direct interaction between ET-1 and tubulointerstitial fibrosis in the UUO model, however, experiments using selective ETA and ETB receptor blockers would be necessary. On the contrary, enalapril increased the mRNA expression of ETB receptor in the obstructed kidney. These data may be very important because ET-1 can promote nitric oxide production by endothelial cells through ETB and implicates another potential mechanism by which ACE inhibitors exert a beneficial effect on renal disease apart from lowering angiotensin II formation. A moderate amount of nitric oxide is beneficial to prevent and alleviate tubulointerstitial fibrosis. It may be worthwhile exploring the role of ETB in the kidney with an obstructed ureter for more details and its interaction among the renin-angiotensin, kinin-bradykinin, and nitric oxide systems.

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REFERENCES


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