Inhibition of aminopeptidase A activity causes an acute albuminuria in mice: an angiotensin II-mediated effect?

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Abstract The hydrolase aminopeptidase A is an important regulator of the renin–angiotensin system, since it inactivates its most vasoactive component angiotensin II (Ang II). A single i.v. injection of a monoclonal antibody against mouse aminopeptidase A (ASD-4) induces a membranous-like glomerulonephritis in mice, characterized by an acute albuminuria, that is not dependent on complement, the coagulation system, or inflammatory cells. We hypothesized that this albuminuria is the consequence of a reduction in aminopeptidase A enzyme activity, that might subsequently lead to an increase in Ang II levels. Aminopeptidase A enzyme activity was analysed in vitro by a fluorimetric enzyme assay and in vivo by enzyme histochemistry. The role of Ang II in the induction of albuminuria in this model was studied by measuring the renal aminopeptidase A mRNA expression in our model by a competitive PCR assay as an indirect measure of Ang II levels. In addition, the role of Ang II in this model was studied by preventing the formation of Ang II with the angiotensin-converting enzyme inhibitor enalapril or by blocking of the Ang II receptor with the ATI receptor antagonist losartan. Only antibodies that were able to inhibit the aminopeptidase A enzyme activity in vitro and in vivo induced an acute albuminuria in mice. Renal aminopeptidase A mRNA expression was increased by injection of the anti-aminopeptidase A antibody. Both enalapril and losartan treatment reduced the acute albuminuria, measured 1 day after injection of a monoclonal antibody against aminopeptidase A, by 91% and 83%, respectively. It is concluded that the induction of acute albuminuria is correlated to the enzyme-inhibiting capacity of the anti-aminopeptidase A antibodies. This impaired enzymatic activity most likely leads to an increase in the levels of Ang II, the best known substrate of aminopeptidase A. The results of our additional experiments are in keeping with our hypothesis that Ang II mediates this acute albuminuria.

Introduction

Aminopeptidase A (APA; EC 3.4.11.7) is a homodimeric, type II integral cell membrane-associated glycoprotein with a molecular weight of approximately 140 kDa of each monomer. APA has a widespread organ distribution, but in the mouse kidney it is predominantly expressed on podocytes and brush borders of proximal tubular epithelial cells [1,2]. In general, APA hydrolyses amino-terminal glutamyl and aspartyl residues from peptides [3]. The best known biological function of this enzyme is related to its effect on the renin–angiotensin system. By cleaving the N-terminal aspartate from angiotensin II (Ang II), APA converts the most active component of the renin–angiotensin system to the less active component Ang III [4]. Apart from this prominent role in the renin–angiotensin system, its biological function is largely unknown [5].

Circulating antibodies can bind to podocytic enzymes such as APA. The interaction of antibodies with their antigenic targets on the cell membranes of podocytes is one of the mechanisms that underlies the formation of subepithelial immune complexes and is a histological hallmark of membranous glomerulonephritis [6]. Recently, we have reported that a single i.v. injection of a monoclonal antibody (mAb) against APA into mice induced a membranous-like glomerulonephritis, that was accompanied by an acute albuminuria. The induction of this acute albuminuria was not dependent on systemic mediators of inflammation known from other experimental models of glomerulonephritis [1]. In view of the fact that APA plays such
a prominent role in the degradation of Ang II, we hypothesized that the binding of the mAb to APA might inhibit the APA enzyme activity, and subsequently might lead to a reduced inactivation and consequent accumulation of Ang II. We speculated that an enhanced or prolonged effect of Ang II on systemic or glomerular haemodynamics, renal cell growth, or renal cytokine production might be responsible for the induction of acute albuminuria in this mouse model. This hypothesis was tested by determining the relationship between the occurrence of albuminuria and the ability of the mAb to inhibit APA enzyme activity in vitro and in vivo. Furthermore, we have measured renal APA mRNA expression as a marker of Ang II activity, and have examined the effects of the angiotensin-converting enzyme (ACE) inhibitor enalapril and the Ang II type 1 AT1 receptor antagonist losartan on the induction of acute albuminuria.

Subjects and methods

Animals

BALB/c and BALB/c, nu/nu mice, weighing 20–25 g and aged 3–6 months, were originally obtained from the Jackson Laboratory (Bar Harbor, ME). All mice were bred in the breeding facility of the central animal laboratory of our university by continuous brother–sister matings.

Monoclonal antibodies

A rat mAb against mouse APA (ASD-4) used for most of the studies was raised as described [1], and propagated as ascites in BALB/c, nu/nu mice. Isolation of the rat mAb from ascites was done by ammonium sulphate precipitation. Seven other rat mAbs against APA used in this study (ASD-2, ASD-3, ASD-37, ASD-38, ASD-39, ASD-41 and ASD-44) were generated and purified in the same way. The concentration of the rat mAb was determined by radial immunodiffusion [7]. The effect of the mAb on the APA enzyme activity was tested in vitro by preincubation of the mAb with a brush border-enriched renal suspension [1].

Enzyme histochemistry

The enzyme activity of APA in the kidneys was visualized by enzyme histochemistry according to Lojda and Gossrau, as described before [2, 8].

APA mRNA in situ hybridization

Parts of the kidneys frozen in liquid nitrogen were used for detection of APA mRNA by RNA in situ hybridization using both a sense and antisense 344 bp digoxigenin-labelled cRNA probe, as described before [9].

Competitive PCR assay for APA mRNA

To compare the expression of APA mRNA under the different experimental procedures, we measured the APA mRNA concentrations extracted from a part of one kidney, with a competitive polymerase chain reaction (PCR) assay.

Construction of competitor molecule

Oligonucleotides were synthesized using the 380B DNA synthesizer (Applied Biosystems, Foster City, USA), and primers were selected on the basis of the mouse APA cDNA sequence [10] using the PRIMER software program (Version 0.5, MIT, Cambridge, MA). For the generation of the competitor molecule as an internal standard a PCR was carried out on the II 1–6 mouse APA cDNA clone (kindly provided by M. D. Cooper, Birmingham, AL [10]) using the following primers: ON-1: 5’-TGG AGA GAG CCT CGG AGT GTT CGT CAG GGG GAG CAA GGG AGC TTC-3’. The ON-2 primer is identical to the 5’ part of ON-14 and complementary to nucleotides 1430 to 1450 of the mouse cDNA sequence, so that both the competitor molecule and the DNA/RNA hybrid reverse transcribed from APA mRNA can be amplified. The final samples of 100 μl were overlaid with two drops of mineral oil. Amplification in a DNA thermal cycler (Perkin Elmer Cetus, Norwalk, CT) started with an initial denaturation step at 94°C for 5 min followed by 40 cycles at 94°C, 42°C, and 72°C each for 1 min. The 509 base pair PCR product was purified using the WIZARD® PCR prep DNA purification system, and analysed on an ethidium bromide-stained 2% agarose gel. The DNA concentration and purity were measured using the Genequant® DNA/RNA calculator (Pharmacia, Uppsala, Sweden).

Competitive assay

Total cellular RNA of half a kidney of mice (n = 5) from the different experimental groups, snap frozen in liquid nitrogen and homogenized with a micropestle, was isolated using the RNAzol B method [11]. The isolated RNA was controlled by agarose gel electrophoresis for integrity, and measured and checked for purity by optical density in a Genequant® DNA/RNA calculator (Pharmacia, Uppsala, Sweden). For the competitive RT-PCR assay 1 μg of isolated total cellular RNA was reverse transcribed for 1 h at 37°C using the ON-1 primer in the following RT mixture (20 μl): 75 mM KCl, 50 mM Tris–HCl pH 8.3, 6 mM MgCl2, 10 mM DTT, 0.2 mM dNTP each, 1.25 μM ON-1, and 4.5 U AMV reverse transcriptase. After completion of the RT reaction 20 μl of a serial dilution in distilled water of the II 1–6 competitor molecule ranging from 1.3 × 10−18 to 9.6 × 10−17 mol were added to these samples. Then 60 μl of PCR mixture were added giving the final concentrations of 65 mM KCl, 20 mM Tris–HCl pH 8.8, 4.8 mM MgCl2, 2 mM DTT, 0.1% NP-40, 0.25 mM dNTP each, 500 nM ON-1, 500 nM ON-2, 5’-TTG AGA GAG CCT CGG AGT GTT CGT CAG GGG GAG CAA GGG AGC TTC-3’. The ON-2 primer is identical to the 5’ part of ON-14 and complementary to nucleotides 1430 to 1450 of the mouse APA cDNA sequence, so that both the competitor molecule and the DNA/RNA hybrid reverse transcribed from APA mRNA can be amplified. The final samples of 100 μl were overlaid with two drops of mineral oil, and after an initial denaturation step at 94°C for 5 min, the mixture was amplified for 35 cycles at 94°C, 55°C, and 72°C each for 1 min. Twenty μl of the PCR product were analysed on an ethidium bromide-stained 2% agarose gel. To control whether the competitive PCR yielded...
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quantitatively reproducible results, a known concentration of the II 1–6 cDNA insert (2.7 × 10⁻¹⁸ mol/reaction) was added instead of a constant concentration of cDNA obtained by RT from total renal RNA (Table 2).

After electrophoresis the agarose gel was uniformly lit by an ultraviolet light source. The fluorescence signal was digitized using a CCD RGB camera with a macro-objective attached to a VIDAS⁺⁺⁺ image analysis system. Using regression analysis the value at log ratio 0 could be calculated, resulting in the value corresponding to the concentration of the mRNA.

**Effect of enalapril and losartan treatment**

The effect of the ACE inhibitor enalapril and the AT1 receptor blocker losartan on the acute albuminuria induced by injection of a mAb against APA (ASD-4) was examined in six groups of BALB/c mice. The effective dosing schedule of enalapril was determined in a pilot study in which we assessed the ability of giving enalapril by gavage or in the drinking water to attenuate serum ACE activity. For losartan, we adopted a comparable dosing schedule. Three groups received 8 mg ASD-4 alone (B), or in combination with enalapril (D) or losartan (F). Three control groups received the same volume of saline instead of ASD-4 alone (A), or in combination with enalapril (C) or losartan (E). Treatment with enalapril (C and D) or losartan (E and F) started 24 h before the administration of ASD-4 or saline by adding 100 mg/l enalapril or losartan to the drinking water and was continued for the following 2 days. Additionally, every 12 h all groups were given 0.5 mg enalapril (C, D) or losartan (E, F), or the same volume of drinking water (A, B) by gavage. Six h after the i.v. injection of ASD-4 or saline, mice were placed in a metabolic cage as described [1,12] and urine was collected during an 18 h period. Twenty-four h after the i.v. injections blood was collected and mice were killed by cervical dislocation, and their kidneys removed and processed for light microscopy, immunofluorescence, RNA *in situ* hybridization, and RNA isolation. Albuminuria, defined as the amount of albumin excreted during 18 h (mean ± SEM), as a sign of glomerular protein leakage, was determined by radial immunodiffusion using a goat anti-mouse albumin antiserum as described [7,12].

**Light microscopy**

Kidney fragments were fixed in Bouin's solution, dehydrated, embedded in paraplast, and 2 μm sections were stained with periodic acid Schiff, and silver methenamine, as described earlier [1].

**Statistical analysis**

For statistical analysis, one-way analysis of variance (ANOVA) was used and group comparisons were done with the Tukey-Kramer test. *P* values <0.05 were regarded as significant. All values are expressed as means ± SEM.

### Table 1. Characteristics of eight mAbs against mouse aminopeptidase A

![Table 1](image)

<table>
<thead>
<tr>
<th>Code mAb</th>
<th>Albuminuria at day 1*</th>
<th>FEA (%)²</th>
<th>Enzyme histochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glomeruli</td>
</tr>
<tr>
<td>ASD-3</td>
<td>9068 ± 2610</td>
<td>8</td>
<td>±</td>
</tr>
<tr>
<td>ASD-4</td>
<td>7494 ± 2877</td>
<td>10</td>
<td>±</td>
</tr>
<tr>
<td>ASD-37</td>
<td>5951 ± 990</td>
<td>18</td>
<td>±</td>
</tr>
<tr>
<td>ASD-39</td>
<td>5948 ± 1343</td>
<td>22</td>
<td>±</td>
</tr>
<tr>
<td>ASD-2</td>
<td>96 ± 34</td>
<td>96</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td>ASD-38</td>
<td>120 ± 46</td>
<td>116</td>
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</tr>
<tr>
<td>ASD-41</td>
<td>133 ± 14</td>
<td>93</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td>ASD-44</td>
<td>120 ± 62</td>
<td>105</td>
<td>+ + + + + +</td>
</tr>
</tbody>
</table>

*For the mAb ASD-37 and ASD-39 20 mg was injected instead of the 10 mg for the other mAb. Data are expressed as μg of albumin excreted per 18 h. *Residual APA activity of a brush border-enriched renal suspension after preincubation with 100 μg of each mAb in the fluorimetric enzyme assay (FEA).

### Results

#### Induction of acute albuminuria

Eight rat mAbs against the mouse hydrolase APA were developed in our laboratory [1]. A single i.v. injection of these mAbs in normal mice resulted in a clear-cut, acute albuminuria using the mAbs ASD-3, ASD-4, ASD-37 and ASD-39, although ASD-37 and ASD-39 had to be given in higher doses (Table 1). The four other mAbs (ASD-2, ASD-38, ASD-41 and ASD-44) never induced albuminuria, even at higher doses (Table 1).

**Effects on APA enzyme activity**

The ability of the anti-APA mAbs to inhibit the APA enzyme activity was tested on a solubilized brush border suspension. The strongly albuminuric mAbs ASD-3 and ASD-4 inhibited the enzyme activity by approximately 90%, the somewhat less effective mAbs ASD-37 and ASD-39 inhibited the enzyme activity by approximately 80%, while the non-albuminuric mAbs ASD-2, ASD-38, ASD-41 and ASD-44 had no effect on the APA enzyme activity *in vitro* (Table 1). ASD-4 was used for further experiments. The effect of this mAb on the renal APA enzyme activity was almost completely inhibited when the mAbs ASD-3, ASD-4, ASD-37 or ASD-39 were injected, while the four non-albuminuric anti-APA mAbs had no effect on the enzyme activity (Table 1). ASD-4 was used for further experiments. The effect of this mAb on the renal APA enzyme activity is illustrated in Fig. 1. One day after injection of the eight mAbs showed that the renal APA enzyme activity was almost completely inhibited when the mAbs ASD-3, ASD-4, ASD-37 or ASD-39 were injected, while the four non-albuminuric anti-APA mAbs had no effect on the enzyme activity (Table 1). ASD-4 was used for further experiments. The effect of this mAb on the renal APA enzyme activity was almost completely inhibited, except for a minute glomerular enzyme activity (Fig. 1A), while no effect on the enzyme activity was seen in saline-injected control mice (Fig. 1B).

### APA mRNA alterations

To further delineate the role of Ang II in the induction of albuminuria in our model we tried to quantitate tissue Ang II levels. Unfortunately, we were not able to measure tissue Ang II levels directly. However, it
Fig. 1. Enzyme histochemistry on frozen kidney sections of a BALB/c mouse 1 day after i.v. injection of 8 mg ASD-4 (A) or saline (B, control). (A) Injection of ASD-4 almost totally abolished the enzyme activity in the entire kidney of the mouse. Only very faint activity can be seen in the glomerulus (x 480). (B) In the control mouse APA activity is present in the glomerulus and in the brush borders of the proximal tubular cells (x 480).

Fig. 2. Expression of APA mRNA in a normal BALB/c mouse kidney by non-radioactive RNA in situ hybridization (RISH). (A) RISH using the antisense riboprobe for APA showed strong cytoplasmic staining of cells located at the periphery of the glomerulus (arrows). Only a faint or negligible staining can be seen in the proximal tubular epithelial cells (x 1000). (B) RISH with the sense riboprobe for APA was completely negative (x 1000).
has been demonstrated that Ang II induces renal APA expression. Kidney APA mRNA levels might therefore provide an indirect measure of renal Ang II. We have determined kidney APA mRNA by in situ hybridization and by a more sensitive technique, the competitive PCR assay.

RNA in situ hybridization. Non-radioactive RNA in situ hybridization with an antisense riboprobe for APA showed strong staining of glomerular cells, and a faint or negligible staining of proximal tubular epithelial cells and smooth muscle cells of arteries in normal mice (Fig. 2A). RNA in situ hybridization at the electron microscopic level demonstrated that in the glomerulus APA mRNA was confined to the podocytes, and not to the endothelial or mesangial cells (data not shown). Hybridizations with the sense riboprobe were negative (Fig. 2B). Using RNA in situ hybridization we did not observe differences in expression between the different experimental groups.

Competitive PCR assay. To examine slight variations in APA mRNA expression in the different experimental groups 1 day after induction of the anti-APA glomerulonephritis, we used a competitive PCR assay that was able to measure attomol levels of APA mRNA from a part of a single kidney of normal mice. To control whether the competitive PCR assay following the RT step gave reproducible, quantitative results, the assay was carried out with the II 1-6 APA cDNA insert of known concentration instead of a constant APA cDNA concentration and the known input cDNA concentration (data not shown). Hybridizations with the sense riboprobe were negative (Fig. 2B). Using RNA in situ hybridization we did not observe differences in expression between the different experimental groups.

Table 2. Pilot experiments to test the accuracy and reproducibility of the competitive PCR

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Number of regression points</th>
<th>Correlation coefficient</th>
<th>cDNA concentration (amol/sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>0.98</td>
<td>2.7</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>0.96</td>
<td>2.7</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>0.98</td>
<td>3.0</td>
</tr>
</tbody>
</table>

To test the accuracy and reproducibility of the competitive PCR assay, 2.7 amol of the II 1-6 cDNA clone, coding for mouse APA, were co-amplified with varying amounts of competitor molecules as described in the Methods. A variation of 4.3% between the measured APA cDNA concentration and the known input cDNA concentration was found.

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Fig. 3. Measurements of APA mRNA levels during induction of acute albuminuria and interventions with enalapril and losartan using a competitive PCR assay. The groups analysed by this competitive PCR assay are the same as in Table 3. Values are expressed as amol/µg of total cellular RNA and are given as means ± SEM. *P<0.05, **P<0.001 compared to group with saline and water.

Reduction of the anti-APA induced acute albuminuria by enalapril and losartan

To further support the hypothesis that Ang II is involved in the induction of acute albuminuria by administration of anti-APA mAb, both the ACE inhibitor enalapril and the AT1 receptor antagonist losartan were given in two separate experiments. Enalapril and losartan treatment was started 1 day before administration of ASD-4 to ascertain that the ACE activity and AT1 receptor were completely blocked at the time ASD-4 was given. One day after administration of ASD-4, the albuminuria induced by ASD-4 was reduced by 91% and 83% in the enalapril and losartan-treated mice, respectively (Table 3). The albuminuria

Table 3. Effect of enalapril and losartan treatment on the acute albuminuria at day 1 after injection of ASD-4

<table>
<thead>
<tr>
<th>Group</th>
<th>Enalapril</th>
<th>Losartan</th>
<th>ASD-4 Albuminuria (µg/18 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>16</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>C</td>
<td>11</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>11</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E</td>
<td>10</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>F</td>
<td>10</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

1 Eight mg of ASD-4 or the same volume of saline (controls) was given i.v. 2 Enalapril or losartan was given by drinking water (100 mg/l) and every 12 h by gavage (0.5 mg). Control mice received the same volume of drinking water by gavage. 3 Mean ± SEM. 4P<0.005 compared to group A.
of mice of the control groups, given saline with or without enalapril or losartan, did not exceed the upper limit of the physiological albuminuria (<100 µg/18 h). (Immunohisto-) histological findings. By light microscopy, mice treated with ASD-4 showed no glomerular abnormalities. The cells of the convoluted and straight parts of the proximal tubules were finely vacuolated and occasionally protein casts were seen. These were absent in ASD-4-injected mice treated with enalapril and losartan (data not shown). Immunofluorescence at 1 day after injection showed binding of ASD-4 in all experimental groups to the glomerular capillary wall in a homogeneous or fine granular pattern as reported earlier [1]. Treatment with enalapril or losartan had no effect on this binding of ASD-4.

Discussion

In several forms of experimental membranous glomerulonephritis, induced by the binding of antibodies to intrinsic antigens on podocytes, it has been shown that podocytes are not just innocent bystander cells.

The interaction of antibodies with their targets on the podocytes induces the release of several pro-inflammatory and vasoactive substances that can influence glomerular permeability and play a role in the progression of the glomerular lesions [6,13]. One of these podocytic antigens in experimental forms of membranous glomerulonephritis is the hydrolase APA. Recently, we have reported that injection of a mAb against APA in mice induced a membranous-like glomerulonephritis that was characterized by a dose-dependent, acute albuminuria. However, in contrast to most other experimental models, this acute albuminuria was not mediated by an inflammatory response [1]. Theoretically, several other mechanisms might be involved in the induction of the acute albuminuria in our model. First, binding of the mAb to the podocyte might trigger the release of one or more mediators, that are capable of altering glomerular permeability. Second, albuminuria might be related to an inhibited activity of APA, and subsequently reduced degradation of one of its substrates. In the present study we have focused on this latter possibility.

Our data clearly demonstrate that only the mAbs that inhibit APA enzyme activity can induce albuminuria. This finding suggests that APA is not merely a target on the podocyte but that the APA enzyme activity plays a crucial role in the induction of acute albuminuria in this model. The involvement of an APA substrate molecule seems likely. However, only a few possible substrates have been identified. The best known substrate of APA is Ang II, the most active component of the renin-angiotensin system. Ang II of endogenous or exogenous origin has long been known to increase urinary protein excretion in various experimental settings [14], which can be explained by a marked elevation of the glomerular capillary pressure and/or systemic blood pressure. Alternatively, increased local or systemic Ang II levels might activate renal cells to release growth factors and cytokines, that indirectly can modulate the glomerular permeselectivity.

To confirm that inhibition of APA enzyme activity causes proteinuria by increasing Ang II levels, we tried to quantitate tissue Ang II levels. Unfortunately, we were unable to directly measure mouse tissue Ang II levels. Therefore, we concentrated on methods which indirectly reflect Ang II levels, such as measurements of the Ang II receptor or expression of renin or APA [15–19]. It has been shown that increasing Ang II levels by either exogenous (infusion of Ang II) or endogenous routes (rat remnant kidney model) increases the renal APA expression [17–19]. Enzyme histochemistry cannot be used in our model since ASD-4 completely blocks the APA enzyme activity in vivo. By in situ hybridization we observed a clear APA mRNA expression in the podocytes, but only faint expression in the proximal tubular epithelial cells. Such a difference in tubular and glomerular APA mRNA expression has also been observed in the rat [20]. This finding is not in correspondence with the immunoreactive pattern shown in earlier studies [1,2,21]. This difference is certainly not due to alternative splicing phenomena or different isoforms, since only a single APA mRNA species can be identified in mouse tissues [2,21]. The difference between immunoreactivity and APA mRNA expression might be explained by a low turnover of APA in the proximal tubular epithelial cells, when compared to the podocytes. In situ hybridization proved insensitive to detect changes in APA mRNA expression. Therefore, we measured the APA mRNA expression by a sensitive, competitive PCR assay that produced accurate and reproducible results. The administration of ASD-4 resulted in a 1.7-fold increase in APA mRNA expression, which is in line with our hypothesis that blockade of APA enzyme activity increases the Ang II concentration. Enalapril, which reduces the generation of Ang II, lowered the APA mRNA expression. The ATI receptor blocker losartan when given alone increased APA mRNA expression. This result is in agreement with the increase in Ang II levels which is invariably seen during ATI receptor blockade [22]. This also indicates that the Ang II-induced increase of APA expression is not mediated through the ATI receptor. Our observations fit with the findings that the ATI receptor is located on mesangial cells and spatially separated from APA, which is localized only on the podocytes. This is also supported by the finding that the expression of APA in podocytes is mediated by increases in intracellular cAMP, a second messenger that is not coupled to the ATI receptor [23,24].

We further examined the role of Ang II in our model by preventing its generation by ACE inhibition with enalapril. This drug has successfully been used as an anti-hypertensive and anti-proteinuric agent in humans and experimental animal models [25]. However, the effects of enalapril may not solely have been due to inhibition of the generation of Ang II; enalapril also retards the breakdown of the vasodilator bradykinin, which may lead to a decrease of blood pressure and
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