Angiotensin-converting enzyme (ACE) null mice display aberrant renal pathology. Inadequate formation of angiotensin II (Ang II) results in hypotension, loss of fluid homeostasis, lack of urine concentration, and failure to regulate GFR through the tubuloglomerular feedback (TGF) mechanism. For examining the tissue-specific role of ACE in renal structure and regulation of renal filtrate formation, single-nephron GFR, proximal tubular fluid reabsorption, and TGF responsiveness were determined in mice that expressed ACE in only one tissue. Maximum TGF responses in mice that expressed somatic ACE (sACE) in proximal tubule cells (Gs strain) or germinal ACE in the serum (Pg strain) were reduced significantly compared with wild-type (WT) mice. In contrast, TGF responses in mice that expressed sACE in vascular endothelial cells (Ts strain) were not different from control. Single-nephron GFR was reduced in Ts compared with WT mice, but fractional reabsorption and therefore glomerulotubular balance were not distinguishable. BP responses to exogenous Ang I were diminished in Ts, Gs, and Pg mice, whereas those to Ang II were the same in the different strains. Plasma and renal tissue Ang I of all transgenic mouse strains was significantly higher than WT, whereas Ang II levels were generally lower; aldosterone levels were significantly lower than WT in Ts mice but not in the two other transgenic strains. Our results demonstrate that vascular expression of sACE can largely but not completely restore TGF regulation of GFR. Proximal fluid reabsorption in the chronic absence of proximal tubule ACE is normal.

Control of fluid homeostasis by the kidneys requires the participation of numerous paracrine agents that act in concert to regulate the formation of a large glomerular ultrafiltrate and the subsequent retrieval of essential constituents along the tubular system. It has been recognized that among these regulatory pathways the renin-angiotensin-aldosterone system (RAAS) seems to play an important and perhaps dominant role (1). Both angiotensin II (Ang II) as the end product of the enzymatic actions of renin and angiotensin-converting enzyme (ACE) and aldosterone, which is secreted in response to Ang II, have profound effects on the absorption of NaCl and water in most segments of the renal nephron. In addition, Ang II is a vasoactive agent that affects GFR by vasoconstrictor actions in glomerular arterioles (2). Tubular absorption and glomerular filtration are linked by the tubuloglomerular feedback (TGF) mechanism. As NaCl concentration rises in the tubular fluid passing the macula densa, for example as a result of a reduction in proximal fluid absorption, vasoconstriction of afferent arterioles occurs, and the resultant change of GFR limits the delivery of fluid to the late nephron (3). It is thought that this tubulovascular cross-talk assists in the preservation of electrolyte balance by limiting sodium excretion. Whereas adenosine acting through A1 adenosine receptors seems to be the direct mediator of this response, a substantial body of evidence indicates that the formation of Ang II is necessary for adenosine to exert its actions (4).

Studies of the effects of pharmacologic inhibitors of the RAAS have been used widely to assess its role in physiology and pathophysiology. This approach was complemented recently by the development of mutant mouse strains defective in the expression of renin, renin substrate, ACE, or angiotensin receptors (5–9). The operation of the RAAS as a single functional unit is supported by the findings that all of these strains have essentially identical abnormalities, including arterial hypotension, renal cortical and papillary atrophy, and hyperplasia of the renal vasculature. Specifically, ACE null mice that are generated in our laboratory in the FVB background have identical renal defects: Hypotension and male sterility (10). In addition, Ace−/− mice are runty and have a life expectancy of only 6 to 8 mo as a result of the severe renal defects. Although the occurrence of these abnormalities is highly informative in itself, the severity of the RAAS null phenotype limits the usefulness of these animals for physiologic studies.
In addition, it has become clear that the RAAS fulfills both endocrine and paracrine roles through the operation of systemic generation of Ang II on the one hand and the local formation of Ang II in certain tissues on the other hand (11). Both the use of pharmacologic inhibitors and the generation of null mutants are unable to distinguish between these two modes of RAAS operation. To address this question, we produced several strains of mice with tissue-specific expression of ACE with the expectation that such animals would facilitate the distinction between systemic and local RAAS actions. The general strategy was to use tissue-specific promoters to drive the expression of a single ACE isomorph in mice with an ACE null FVB background (10,12,13).

The main goal of this study was to assess the effect of selective expression of somatic ACE (sACE) in vascular endothelial cells (Ts strain). For comparison, TGF responses were also determined in mice with expression of sACE in renal proximal tubule cells using the γ-glutamyl transpeptidase promoter (Gs strain) and in a strain of mice that expresses germinal ACE (gACE) in sperm using the human PGK2 promoter (Pg strain). Mice of the Ts and Gs strains have sACE in their serum as a result of the natural cleavage-secretion processing of membrane-anchored ACE (13), whereas Pg mice ectopically express gACE in the serum (10). Our observations in the Ts strain show that TGF regulation was largely but not entirely normalized by expression of ACE in vascular endothelial cells. Furthermore, the absence of ACE in renal proximal tubule cells does not cause measurable changes of fluid reabsorption. These results further support the notion that specific physiologic functions of ACE are determined by the location of ACE expression. Moreover, the overexpression of ACE in proximal tubules is redundant in the sense that its long-term absence does not compromise proximal NaCl and fluid reabsorption.

**Materials and Methods**

**Mice**

The FVB strains of mice that were used in this study have been characterized previously (10,13) and have the following genotypes: Ts (Ace−/−, Ts+/+), Gs (Ace−/−, Gs+/−) and Pg (Ace−/−, Pg+/−). Animals were maintained on a standard rodent diet and sterile tap water, and studies were performed at the age of 4 mo.

**Aldosterone and Ang I and II Levels**

Plasma aldosterone levels were determined as described previously (14) in four age-matched adult mice of each genotype using the Coat-A-Count method according to the manufacturer’s instructions (Diagnostic Products Corp., Los Angeles, CA). The genotype averages that were obtained by duplicate assays from each mouse are reported ±95% confidence interval (CI). Plasma and renal Ang I and Ang II levels both were measured as described previously (13,15). Duplicate assays on a minimum of three plasma pools, each pool obtained from four to five age-matched mice, were averaged and reported ±95% CI. Duplicate assays on the kidneys from five mice of each genotype were averaged and reported ±95% CI.

**GFR and Renal Blood Flow**

For determining GFR, mice were infused with 125I iothalamate (Glofil, Questcor Pharmaceutical, Hayward, CA) at approximately 5 μCi/h. After 30 to 45 min of equilibration, a blood sample of approximately 4 μl was collected into heparinized 5-μl microcaps (Drummond, Broomall, PA), and a urine collection was made for 30 min following by a second blood collection. 125I iothalamate radioactivity was measured in duplicate 0.5-μl aliquots of plasma and urine in a RiaStar γ-counter (Packard Instrument Co., Downers Grove, IL). Urine volume was determined gravimetrically. Measurements of renal blood flow (RBF) were performed in a separate group of wild-type (WT) and Ts mice. The left renal artery was approached from a flank incision and carefully dissected free to permit placement of a 0.5PSB nanoprobe connected to a T402-PB flow meter (Transonic Systems, Ithaca, NY). The probe was held in place with a micromanipulator. The flow signal was digitized and analyzed using PowerLab software (ADInstruments, Colorado Springs, CO). RBF was determined for 20 min and values given represent the average for the observation period.

**Micropuncture Studies**

The preparation of mice and the techniques that were used to assess proximal tubule function and TGF responses of stop flow pressure have been described in detail (16). In short, the left kidneys of mice under inactin/ketamine anesthesia were approached from a flank incision and placed in a Lucite cup adapted for the size of the mouse kidney. Neophron filtration and absorption rates were determined from timed end-proximal fluid collections using 125I iodide as a volume marker. TGF responses were assessed as the change of stop flow pressure (Psf) in response to an increase of loop of Henle perfusion rate from 0 to 30 nl/min. The perfusion fluid contained (in mM/L) 136 NaCl, 4 NaHCO3, 4KCl, 2CaCl2, and 7.5 urea and 100 mg/100 ml FD&C green (Keystone).

**Immunohistochemistry**

Adult male mice were killed by inhalation of 100% CO2. Histochoice-preserved, paraffin-embedded kidney sections were prepared as described previously (13). The monoclonal mouse anti-rabbit ACE antibody 3C5 (Mono-ACE, River Forest, IL), which recognizes rabbit ACE but not mouse ACE, was applied at 1:25 in PBST blocking solution (17). After washes with PBS at 25°C, goat anti-mouse AlexaFluor 594 (Molecular Probes, Eugene, OR) was applied at a 1:1500 dilution in blocking buffer for 2 h at 25°C. Sections were washed in PBS before mounting with Vectashield + DAPI. Slides were visualized with a Leica fluorescence microscope at ×40 total magnification.

**BP Responses to Angiotensin**

BP was measured through a catheter in the femoral artery, and pressure signals were digitized, recorded, and analyzed using PowerLab software. Responses to Ang I and II were assessed in six WT, seven Ts, five Gs, and four Pg mice by giving bolus injections of freshly prepared peptide solutions through a jugular vein catheter. Peptides were prepared as 1-ng/μl solutions, and volumes of 5, 10, 20, 50, and 100 μl were given to deliver 5, 10, 20, 50, or 100 ng. The same mice were used to establish a full dose-response relationship. After each injection, BP was allowed to return to baseline before a new injection was made.

**Statistical Analyses**

Data are given as arithmetic means and variations as SEM. Significance comparisons were done with ANOVA in combination with the Bonferroni post hoc test or with the t test as appropriate.
Results

Phenotypic Profile of Experimental Mice

The transgenic mice that were used in this study have been previously characterized (10,13), and their phenotypes, compared with WT or ACE null mice, are summarized in Table 1. In those studies, only the Ts strain that expressed ACE in vascular endothelial cells was found to have WT BP levels, despite the absence of proximal tubule ACE expression. Circulating ACE of either germinal or somatic isoform was sufficient to restore gross renal structure, function, health, and blood vessel wall architecture (arteriole thickness). Male fertility was restored in the Pg strain, in which germinal ACE is expressed on the surface of sperm (10,18). Renal ACE levels in all strains were intermediate between WT and ACE null mice. Despite WT serum ACE levels in both Gs and Pg strains, BP was not fully restored. Male fertility was restored only in Pg mice that expressed germinal ACE in sperm.

Immunohistochemistry

ACE expression in the kidney was determined by immunohistochemical staining using the 3C5 mAb that binds only to transgene-expressed rabbit ACE (Figure 1, B through D) but not WT murine ACE expressed in the vascular endothelium and proximal tubules (Figure 1A). sACE staining in the Gs strain was observed in the S1 region of the proximal tubules nearest the Bowman’s capsule of the glomerulus (Figure 1B). Staining of gACE in the Pg strain was localized to the S1 region of a very small number of renal proximal tubules but was observed primarily in serum flowing through glomerular capillaries and in the blood of larger vessels (Figure 1C). In the Ts strain, sACE expression is restricted to the vascular endothelial cells of blood vessels and in the serum (Figure 1D).

Endogenous Hormone Levels

We determined the endogenous level of Ang I and Ang II in the kidneys and plasma of WT and transgenic strain mice. Figure 2 shows that the level of Ang II was decreased in the kidneys of all transgenic strains (pg Ang II/g kidney), significantly so in the Ts strain (WT = 721 ± 100, Ts = 369 ± 37, Gs = 437 ± 30, Pg = 539 ± 39). A significant reduction in the plasma Ang II (pg Ang II/ml) in the Ts and Pg strains (WT = 85.2 ± 655) was observed. Conversely, the level of Ang I was significantly elevated in the kidney (pg Ang I/g kidney; WT = 250 ± 34, Ts = 655 ± 27, Gs = 395 ± 46, Pg = 386 ± 39) and the plasma (pg Ang I/ml; WT = 222 ± 53, Ts = 969 ± 85, Gs = 572 ± 79, Pg = 1221 ± 119) of all transgenic strains. This result illustrates the partial restoration of Ang II production as a result of tissue-restricted expression of ACE in each transgenic strain. Because Ang II stimulates aldosterone release by the adrenal cortex, we also measured the plasma aldosterone levels in all experimental groups. As shown in Figure 3, the aldosterone level in the Ts and Pg strains but not the Gs strain were significantly different from WT strain mice (ng aldosterone/ml plasma; WT = 2.7 ± 0.2, Ts = 2.3 ± 0.1, Gs = 2.6 ± 0.2, Pg = 3.1 ± 0.1).

Table 1. Phenotype summary of mouse strains

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>KO</th>
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<td>=</td>
<td>=</td>
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<td>=</td>
<td>=</td>
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<tr>
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<td>↑↑</td>
<td>=</td>
<td>=</td>
<td>=</td>
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<tr>
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<tr>
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<td>=</td>
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<tr>
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<td>↓</td>
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<tr>
<td>Blood pressure</td>
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KO, knockout; Ts, Ace−/−, Ts +/+; Gs, Ace−/−, Gs +/+; Pg, Ace−/−, Pg +/+; =, equal to wild-type; ACE, angiotensin-converting enzyme.

BP Response to Angiotensin

To functionally assess ACE activity in the vascular system, we determined the acute BP response to intravenous bolus injections of Ang I. We also determined BP responses to Ang II to compare the effect of angiotensin at the receptor and postreceptor level. Data are summarized in Figure 4. It can be seen that the response to Ang I was reduced in all transgenic mice, particularly at lower doses of injected Ang I. For example, at a dose of 10 ng, Ang I increased BP by 22 ± 2.7 mmHg in WT but only by 3.2 ± 0.7 mmHg in Ts, by 5.4 ± 2.5 mmHg in Pg, and by 7.7 ± 0.8 mmHg in Gs mice. Responses were significantly lower in all transgenic mice compared with WT but were not different between transgenic animals. In contrast, responses to
Ang II were comparable and not different between all animals tested.

**TGF Response**

TGF responses were determined in five WT, five Ts, five Pg, and four Gs mice. The gender of all animals was male. There were no significant differences in body weight among WT, Ts, Pg, and Gs mice (31 ± 1.0, 30 ± 0.8, 28 ± 1.7, and 30 ± 0.9 g, respectively). Similarly, kidney weights of the micropunctured left kidneys were not different between genotypes (194 ± 4.7, 239 ± 11, 229 ± 18, and 217 ± 12 mg). \( P_{\text{SF}} \) without perfusion of the loop of Henle averaged 39.4 ± 0.9 mmHg in WT \((n = 17)\), 42.6 ± 3.1 mmHg in Ts \((n = 17)\), 42.1 ± 1.3 mmHg in Pg \((n = 17)\), and 39 ± 1.5 mmHg in Gs \((n = 17)\). Differences were not statistically different. In response to an increase in loop of Henle perfusion to 30 nl/min, a flow rate that is known to saturate the TGF response in mice, \( P_{\text{SF}} \) fell significantly in all genotypes, by 8.1 ± 0.9 mmHg in WT, by 7.1 ± 0.9 mmHg in Ts, by 4.5 ± 0.8 mmHg in Pg, and by 5.0 ± 0.43 mmHg in Gs mice (Figure 5). Changes in \( P_{\text{SF}} \) were significantly smaller than control in Pg and Gs strains when tested by ANOVA with Bonferroni post hoc test \((P < 0.01 \text{ and } P < 0.05, \text{ respectively})\). Mean arterial BP of anesthetized mice for the period of the micropuncture procedure averaged 113 ± 1.2 mmHg in WT, 93 ± 2.5 mmHg in Ts, 98 ± 1.4 mmHg in Pg, and 89 ± 2.0 mmHg in Gs mice. Mean BP of all genetically variant mice under anesthesia were significantly lower than WT mice at \( P < 0.001 \) (ANOVA). Among the transgenic mice, BP in Gs mice was significantly lower than in animals of the Pg strain \((P < 0.01)\).

**Kidney GFR and RBF**

Kidney GFR averaged 476 ± 47 ml/min in WT mice \((n = 4)\) and 427 ± 77 ml/min \((n = 4)\) in Ts mice, a filtration rate that is not significantly different from WT. RBF was 1.09 ± ml/min in WT and 0.83 ± 0.21 ml/min in Ts mice at mean arterial pressures of 86 ± 11 and 87 ± 5.7 mmHg, respectively.

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*Figure 2.* Mean plasma and renal angiotensin I (Ang I) and Ang II levels in WT mice and in three transgenic ACE-expressing lines \((n = 5 \text{ for all genotypes})\). Error bars indicate 95% confidence interval (CI), and significances are given in comparison with WT mice by unpaired \( t \) test \((^*P < 0.01, ^{**}P < 0.001, ^{***}P < 0.0001, ^{****}P < 0.00001, ^{*****}P < 0.0000001\)).

*Figure 3.* Mean plasma aldosterone levels in WT and in three transgenic ACE-expressing lines \((n = 4 \text{ for all genotypes})\). Error bars indicate 95% CI, and significances are given in comparison with WT mice by unpaired \( t \) test \((^*P < 0.01, ^{**}P < 0.05)\).
Single-Nephron GFR and Tubular Absorption

Single-nephron GFR (SNGFR) and rates of proximal tubular reabsorption were determined in four WT (FVB strain; Taconic) and four Ts transgenic animals using standard free-flow micropuncture. SNGFR averaged 9.4 ± 0.9 nl/min in WT (n = 16) and 7.7 ± 0.38 nl/min in Ts mice (n = 34; P = 0.043 by unpaired t test). Individual measurements of SNGFR in the two groups of mice are displayed in Figure 6. The reduction of SNGFR is probably related to the lower mean arterial BP in this group of Ts mice (77.5 ± 3.7 versus 98 ± 2.0 mmHg). Proximal fractional absorption of 47.1 ± 3% in WT and 50.3 ± 2.2% in Ts were not significantly different (P = 0.41; Figure 7). Similarly, absolute rates of proximal reabsorption were not different between WT and Ts animals (4.6 ± 0.6 versus 3.9 ± 0.3 nl/min; P = 0.21). As shown in Figure 8, glomerulotubular balance, the relationship between SNGFR and tubular fluid reabsorption in the proximal tubule, was not different between WT and Ts mice.

Discussion

ACE, as well as all other components of the renin-angiotensin system (RAS), is expressed in a variety of organs, including the kidney (16,19,20). The generation of Ang II by the tissue RAS suggests a role of the peptide as a paracrine modulator of organ function in addition to its function as a circulating hormone. Distinguishing between the importance of systemic and locally produced Ang II is not trivial because pharmacologic inhibitors as well as knockout approaches affect both modes of angiotensin action. Our study used transgenic mice that had been genetically engineered to express a single ACE isoform in a cell type–specific location on the background of an ACE null genotype (10,13). This approach seems well suited to assess the notion of a location-specific role of ACE and to determine the contribution of this restricted expression pattern to the overall health and organ function of an animal.

Previous studies in AT1A and ACE knockout mice as well as earlier experiments with ACE inhibitors and angiotensin recep-
Tor blockers have shown that an intact RAS is necessary for the regulatory response of GFR to changes in distal NaCl concentration, the so-called TGF response (3,21). The main focus of this report was to evaluate whether selective expression of ACE in vascular cells is capable of maintaining TGF activity and to compare the role of vascular ACE with that of proximal tubule ACE. In addition, we examined proximal tubular fluid reabsorption in mice that express only vascular ACE but lack ACE activity in proximal tubules. A strain of transgenic mice in which the Tie-1 promoter was used to drive vascular ACE expression in mice with a null mutation of the native ACE gene was generated and previously described (13). In these experiments, we used Ts mice to investigate the specific role of vascular ACE on TGF and proximal tubule function. Functional studies in these mice are greatly facilitated by the lack of the renal deformities that characterize mice with null mutations in any of the RAS components, including the ACE-deficient strains studied to date (6,7,12,13,18).

In confirmation of previous observations in conscious animals, our data show that BP was somewhat reduced in anesthetized mice with selective expression of ACE in the proximal tubule and in mice that expressed only germinal ACE. In addition, anesthetized Ts mice had BP that in two of the three experimental series were slightly but significantly lower than in WT, a finding that is in contrast to previous measurements in the conscious state (13). It is possible that this is a consequence of the anesthesia, and one could speculate that the absence of a functional brain RAS in these Ts strain mice reduces an ACE-dependent component that in WT maintains normal BP during anesthesia. It is to be noted that BP varied considerably between experimental series in a given strain, suggesting that BP during anesthesia and surgery depends more on the response of individual animals to the procedure than on pre-existing pressure levels. Overall, however, BP reductions were not as limiting as they are in ACE and AT1 null mutants. This is also reflected in the fact that Psf at zero loop flow, a variable that is generally dependent on BP, did not vary significantly between WT and transgenic mice.

Our data show that TGF responses were essentially normal in mice that express ACE in the vasculature but that are devoid of membrane-associated ACE expression at all other natural ACE expression sites. Thus, it seems that endothelial ACE is an important contributor to the Ang II required for full TGF responsiveness. The same conclusion was reached in a recent study in which TGF responses were found to be significantly reduced in ACE null mice that do not possess tissue-bound ACE in either vascular or proximal tubules locations and in which a normal BP was achieved by ectopic expression of ACE in the liver (22). In contrast, significant impairment of TGF responsiveness was found in the Gs and Pg strains, presumably as a result of lack of endothelial ACE expression.

Nevertheless, TGF responses in both Pg and Gs strain mice were not abolished, indicating that maintenance of TGF responsiveness, albeit at a reduced level, can be achieved by Ang II generated by plasma ACE. Some contribution of systemically generated Ang II to TGF responsiveness is also supported by previous findings showing that intravenous or peritubular administration of Ang II augmented TGF responses (23,24). Thus, the amount of Ang II that is required for full TGF responsiveness seems to be derived mainly from the action of membrane-associated ACE in endothelial cells, but ACE in the circulating blood stream can contribute to this Ang II pool to some lesser extent. An open question is that of the actual activity of circulating ACE. Previous data have shown that plasma ACE activity determined in vitro by cleavage of a small synthetic substrate was essentially normal in Gs and Pg animals (10,13). However, our experiments showed that BP responses to Ang I were reduced and that the Ang I/Ang II ratio in both kidney and plasma of all transgenic mice was increased, observations consistent with some degree of functional ACE deficiency. We have no satisfactory explanation for this discrepancy other than suggesting that either plasma ACE activity is in fact not the main determinant of acute Ang I conversion or that the use of the synthetic substrate does not reflect Ang I conversion in vivo. It is important to point out the underlying assumption of the

![Figure 7](image_url)

**Figure 7.** Individual measurements of fractional proximal fluid absorption in WT and TS+/+ mice. Horizontal bars indicate mean values. Significance of difference was tested by unpaired t test.

![Figure 8](image_url)

**Figure 8.** Relationship between proximal tubular fluid reabsorption and SNGFR in WT and TS+/+ mice. Lines indicate linear regressions.
above arguments that the overall vascular responsiveness of the vasculature is not altered in the transgenic models used in our study. This assumption is supported by the normal responses of BP to systemically applied Ang II.

Another goal of this study was to assess proximal tubular absorptive function in mice that lack ACE and, therefore, Ang II production in the proximal tubules. Using Ts mice as a model of proximal tubule ACE deficiency, we observed that although the rate of single-nephron filtration was significantly reduced, the relationship between proximal tubular reabsorption and GFR was not significantly different from WT animals. Thus, we conclude that the chronic absence of proximal tubular Ang II production does not exert long-term effects on proximal fluid and NaCl transport. It is possible that Ang II is delivered to the proximal tubule lumen by filtration. However, because proximal tubule Ang II concentrations have been found, at least in the rat, to exceed plasma levels by a factor of approximately 50, it is unlikely that sufficiently high luminal concentrations can be reached by ultrafiltration alone (25,26). The absence of a measurable inhibition of proximal fluid absorption in Ts mice is distinct from the clear reduction of proximal fluid transport that results from acute and local administration of ACE inhibitors or Ang II receptor blockers (27,28). Thus, one has to conclude that changes of Ang II formation in the proximal tubules. Using Ts mice as a model another argument that the overall vascular responsiveness of the vasculature is not altered in the transgenic models used in our study. This assumption is supported by the normal responses of BP to systemically applied Ang II.

We conclude that ACE in vascular tissue is responsible for most of the Ang II generation required for full expression of TGF responsiveness, whereas plasma ACE plays a minor role in contributing to the Ang II pool that affects TGF. Absence of local Ang II generation in the proximal tubule is not associated with long-term effects on proximal tubular fluid absorption.

Acknowledgments

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