Micropuncture determination of nephron function in mice without tissue angiotensin-converting enzyme

Seiji Hashimoto,1 Jon W. Adams,2 Kenneth E. Bernstein,2 and Jurgen Schnerramm1

1National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland; and 2Department of Pathology, Emory University, Atlanta, Georgia

Submitted 10 August 2004; accepted in final form 9 October 2004

Hashimoto, Seiji, Jon W. Adams, Kenneth E. Bernstein, and Jurgen Schnerramm. Micropuncture determination of nephron function in mice without tissue angiotensin-converting enzyme. Am J Physiol Renal Physiol 288: F445–F452, 2005. First published October 19, 2004; doi:10.1152/ajprenal.00297.2004.—To determine the role of the local renin-angiotensin system in renal function, micropuncture was performed on two lines of mice in which genetic changes to the angiotensin-converting enzyme (ACE) gene markedly reduced or eliminated the expression of renal tissue ACE. Whereas blood pressure is low in one line (ACE 2/2), it is normal in the other (ACE 1/3) due to ectopic hepatic ACE expression. When normalized for renal size, levels of glomerular filtration rate [GFR; μl·min⁻¹·g kidney wt⁻¹ (KW)] and single-nephron GFR (SNGFR; nl·min⁻¹·g KW⁻¹) were similar between wild-type (WT) and ACE 1/3 mice, while both measures were significantly reduced in ACE 2/2 mice (WT: 500 ± 63 and 41.7 ± 3.5; ACE 1/3: 515.8 ± 71 and 44.3 ± 3.3; ACE 2/2: 131.4 ± 23 and 30.3 ± 3.5). Proximal fractional reabsorption was not significantly different between WT and ACE 1/3 mice (51 ± 3.5 and 49 ± 2.3%), and it was increased significantly in ACE 2/2 mice (74 ± 3.5%). Infusion of ANG II (50 ng·kg⁻¹·min⁻¹) increased mean arterial pressure by ~7 mmHg in all groups of mice and reduced SNGFR in WT and ACE 1/3 mice (to 30.9 ± 2.8 and 31.9 ± 2.5 nl·min⁻¹·g KW⁻¹) while increasing it in ACE 2/2 mice (to 55.3 ± 5.3 nl·min⁻¹·g KW⁻¹) despite an increase in total renal vascular resistance. The tubuloglomerular feedback (TGF) response was markedly reduced in ACE 1/3 mice (stop-flow pressure change ~2.5 ± 0.9 mmHg) compared with WT despite similar blood pressures (~8.3 ± 0.6 mmHg). In ACE 2/2 mice, TGF was absent (~0.7 ± 0.2 mmHg). We conclude that the chronic lack of ACE, and presumably ANG II generation, in the proximal tubule was not associated with sustained proximal fluid transport defects. However, renal tissue ACE is an important contributor to TGF.

nephron filtration rate; proximal fluid reabsorption; tubuloglomerular feedback; angiotensin II kidney weight

THE RENIN-ANGIOTENSIN SYSTEM has been established to play a critical role in extracellular volume and blood pressure homeostasis. Renin is a proteolytic enzyme that is secreted by the juxtaglomerular granular cells of the kidney and that catalyzes the release of angiotensin I from the renin substrate angiotensinogen (28, 29). Angiotensin I is cleaved by angiotensin-converting enzyme (ACE) to form bioactive angiotensin II (28, 29). There can be little doubt that systemically formed angiotensin II acting in an endocrine fashion exerts important effects throughout the organism. Nevertheless, it has become clear that in addition to the so-called systemic renin-angiotensin system, production of angiotensin II can also occur by enzyme-substrate interactions within certain tissues (11). The most consistent of these tissue renin-angiotensin systems are found in the brain and in the kidney, but such local angiotensin generation appears to be a characteristic of many other tissues as well (9, 12, 22).

Discriminating between the actions of angiotensin II formed systemically or formed locally has been difficult because the standard intervention of administering ACE inhibitors or angiotensin receptor blockers does not distinguish between endocrine or paracrine pathways. Nonconditional gene deletions have been helpful in delineating the role of the renin-angiotensin system in developmental and global functional aspects but have been unable to address the question of specific, local roles of angiotensin II depending on its place of origin (17, 18). Definitive progress has been made with mouse models in which a nonnative renin substrate has been expressed in proximal tubules exclusively and in which mice with this transgene have been found to develop arterial hypertension (8, 10). Since plasma angiotensin II levels were found to be normal, and high levels of the imported substrate were retrieved in the urine, it appeared as if local generation of angiotensin II in the proximal tubule was responsible for the blood pressure aberration (8, 10).

These observations are in agreement with the evidence that proximal tubules can generate renin substrate and, catalyzed by locally produced or filtered renin, can produce angiotensin I (23). Angiotensin I can be converted to angiotensin II by ACE that is abundantly expressed in the brush border of proximal tubules (6, 35, 37). It has been suggested therefore that proximal tubular reabsorption may be under the control of angiotensin II formed locally in the lumen of the proximal tubule and that such a local action may therefore be responsible for some of the hypertensinogenic actions of the peptide (19, 26).

The present experiments were performed to further study the role of the local renin-angiotensin system in the kidney in controlling proximal tubule function and tubuloglomerular feedback (TGF) responsiveness. Previous studies have shown that deletion of the COOH terminus of ACE generates mice with plasma ACE but without any tissue-associated ACE (ACE 2/2 mice) (14). This ACE 2/2 mice lack renal brush border-associated ACE and therefore are incapable of forming angiotensin II in the proximal tubule. Since most of total body ACE activity is represented by enzyme anchored in the membrane of endothelial and epithelial cells, complete elimination of tissue ACE was associated with a marked reduction of blood pressure and a deficiency in overall renin-angiotensin system function.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
(14). The blood pressure abnormality has been corrected in a second line of genetically modified mice in which the ACE gene was ectopically expressed in the liver under the control of the albumin promoter (ACE 3/3). These mice have normal blood pressure despite the lack of all endothelial ACE (6). In the present study, we used a compound heterozygote variant of this strain in which one ACE allele is null and the other ACE allele targets ACE expression to the liver (ACE 1/3) (7). These mice have normal blood pressure and, like the ACE 2/2 and ACE 3/3 mice, lack expression of ACE in the endothelium. The ACE 1/3 mice have only 6–7% the levels of renal ACE found in wild-type (WT) animals. These very low levels of ACE are detectable throughout the proximal tubule, as opposed to WT mice in which ACE expression increases toward the S3 segment of the proximal tubule (6).

The main goal of the present study was to determine rates of nephron filtration and proximal reabsorption in WT mice and in mice without tissue ACE. Using the micropuncture technique in WT, ACE 2/2, and ACE 1/3 mice, we found that the absence of tissue ACE, and therefore of ACE in the proximal tubule brush border, did not significantly affect the relationship between glomerular filtration rate (GFR) and reabsorption. Thus these observations do not yield evidence that the chronic lack of local production of angiotensin II is associated with major changes in proximal reabsorption under basal conditions.

METHODS

Animals. All animal studies were performed according to protocols examined and approved by the Animal Use and Care Committee of the National Institute of Diabetes and Digestive and Kidney Diseases. Experiments were performed in three strains of mice: homozygous ACE 2/2, ACE 1/3, and WT mice. For studies with ACE 2/2 mice, animals were generated from the mating of ACE 2 heterozygous mice. This mating also produced WT littersmate controls. ACE 1/3 mice were produced by mating heterozygous ACE.1 mice with homozygous mutant ACE.3 mice (13). All genotype determinations were made as previously described. All mice used in this study were male. They varied in age from 8 to 24 wk.

The animals were maintained on standard rodent chow and tap water. Mice were anesthetized with Inactin (100 mg/kg ip) and ketamine (100 mg/kg im). Body temperature was maintained at 38°C by placing the animals on an operating table with a servo-controlled heating plate. The trachea was cannulated, and a stream of 100% O2 was blown toward the tracheal tube throughout the experiment. The jugular vein was cannulated with two hand-drawn polyethylene catheters, one for an intravenous maintenance infusion of 2.25 g/100 ml bovine serum albumin in saline at a rate of 0.5 ml/h and the other for arterial blood pressure and blood withdrawal. Another bovine serum albumin in saline at a rate of 0.5 ml/h and the other for jugular vein was cannulated with two hand-drawn polyethylene catheters.

RESULTS

Body and kidney weights. Mean body weights were 39.7 ± 1.1 g in WT (n = 9), 41.3 ± 3 g in ACE 2/2 (n = 7; not significant), and 27.8 ± 0.5 g in ACE 1/3 mice (n = 11; P < 0.001 compared with WT). Kidney weights were 584 ± 17.5 mg in WT and 392 ± 30.5 and 378 ± 11.5 mg in ACE 2/2 (P < 0.001) and ACE 1/3 (P < 0.001) mice, respectively. Thus, while body weights of WT and ACE 2/2 mice were the same, kidney weights were significantly lower in ACE 2/2 than control mice. The relationship between body weight and kidney weight is shown in Fig. 1. It can be seen that body and kidney weights of ACE 1/3 mice were lower than of WT animals but that the relationship between body weight and kidney weights was the same in the two strains of mice. In contrast, the kidney weights of ACE 2/2 mice were consistently lower for any given body weight than in either WT or ACE 1/3 mice.

Hemodynamic response to angiotensin. Responses of arterial blood pressure and renal vascular resistance to bolus injections of angiotensin II (10 and 50 ng) and angiotensin I (13 and 65 ng) were determined in five WT, five ACE 2/2, and five ACE 1/3 mice. Mean arterial blood pressure in response to angiotensin II increased in these three groups of mice with comparable sensitivity, whereas blood pressure changes in
response to angiotensin I were virtually absent in ACE 2/2 and diminished in ACE 1/3 mice (Fig. 2). The reduced blood pressure response to angiotensin I in the ACE 1/3 mice is consistent with the original publication on these animals (7). This probably reflects the high circulating angiotensin I levels found in these mice and the reduced total levels of ACE (7). These determinations, combined with measurements of RBF by using ultrasonic flowmeters, permitted calculation of the response of renal vascular resistance to angiotensins. Renal resistance increased significantly in all groups of mice in response to angiotensin II although the increase was less in ACE 1/3 mice. As expected, angiotensin I had markedly lower effects on renal vascular resistance in ACE 2/2 and ACE 1/3 than WT mice (Fig. 2).

GFR and RBF. RBF (1 kidney), as determined with an ultrasonic flowmeter, averaged 1.14 ± 0.08 ml/min in WT (n = 5), 0.77 ± 0.1 ml/min in ACE 2/2 (n = 5), and 1.27 ± 0.13 ml/min in ACE 1/3 mice (n = 5; Fig. 3). Mean arterial blood pressures in these anesthetized and laparatomized animals were 97 ± 7.4, 48 ± 5.5, and 84 ± 4 in WT, ACE 2/2, and ACE 1/3 mice, respectively. Renal vascular resistance tended to be lower in ACE 2/2 mice, but differences between genotypes were not significant. The effect of an intravenous infusion of angiotensin II at 3 ng·kg⁻¹·min⁻¹ on renal hemodynamics is included in Fig. 3. It can be seen that angiotensin II decreased RBF and increased renal vascular resistance in WT mice as well as in ACE 2/2 and ACE 1/3 mice.

GFR expressed both in absolute terms and normalized for kidney weight is summarized in Fig. 4. As can be seen, GFR was not significantly different between WT (500 ± 63 µl·min⁻¹·g kidney wt⁻¹ and 294.6 ± 39.6 µl/min; n = 9) and ACE 1/3 mice (515.8 ± 71 µl·min⁻¹·g kidney wt⁻¹ and 184.3 ± 24.2 µl/min; n = 6) when tested by ANOVA with a Bonferroni correction. The tendency for the lower absolute GFR in the ACE 1/3 mice probably reflects their lower kidney weight. In contrast, the GFR in ACE 2/2 mice was significantly reduced compared with WT or ACE 1/3 when expressed both in absolute and kidney weight-corrected terms (131.4 ± 23 µl·min⁻¹·g kidney wt⁻¹ and 53.6 ± 13.6 µl/min; n = 7).
Mean arterial blood pressure in this group of animals again was significantly lower in ACE 2/2 mice than in either WT or ACE 1/3 animals (63 ± 4 compared with 95 ± 4.5 and 85 ± 4 mmHg). As also shown in Fig. 4, infusion of angiotensin II reduced GFR in WT mice to 385 ± 99 μl·min⁻¹·g kidney wt⁻¹ (P = 0.03 vs. control) and in ACE 1/3 mice to 360 ± 47 μl·min⁻¹·g kidney wt⁻¹ (P = 0.02 vs. control), whereas it increased GFR in ACE 2/2 mice to 179 ± 31 μl·min⁻¹·g kidney wt⁻¹ (P = 0.007 vs. control). Mean arterial blood pressure during the infusion of angiotensin II increased by about 6–7 mmHg in all groups.

*Single-nephron GFR and tubular reabsorption.* Single-nephron GFR (SNGFR) and rates of proximal tubular reabsorption were determined in five WT, four ACE 2/2, and five ACE 1/3 transgenic animals using standard free-flow micropuncture. Mean body weight of the mice used in the micropuncture studies was 42 ± 1 g in WT mice, 37.2 ± 1.6 g in ACE 2/2, and 27.8 ± 1.07 g in ACE 1/3 mice (P < 0.001 compared with either WT or ACE 2/2, ANOVA). Left kidney weights averaged 306 ± 23.1 mg in WT, 185 ± 11 mg in ACE 2/2 (P < 0.001 compared with WT), and 177 ± 4 mg in ACE 1/3 (P < 0.001 compared with WT). SNGFR (Fig. 5) averaged 11.7 ± 0.9 nl/min in WT (n = 14), 4.9 ± 0.6 nl/min in ACE 2/2 (n = 12; P < 0.001 compared with WT, ANOVA), and 7.8 ± 0.6 nl/min in ACE 1/3 mice (n = 23; P < 0.001 compared with WT, P < 0.05 compared with ACE 2/2, ANOVA). As also shown in Fig. 5, the difference in SNGFR between WT and ACE 1/3 mice was abolished by kidney weight correction (41.7 ± 3.5 nl·min⁻¹·g kidney wt⁻¹ vs. 44.3 ± 3.4 nl·min⁻¹·g kidney wt⁻¹), whereas SNGFR remained lower in ACE 2/2 mice (30.3 ± 4 nl·min⁻¹·g kidney wt⁻¹; P < 0.05 compared with WT, ANOVA).

The response of SNGFR to an infusion of angiotensin II consisted of a significant reduction in both WT and ACE 1/3 mice to 8.6 ± 0.7 and 5.7 ± 0.4 nl/min, respectively (P = 0.01 and P = 0.008 compared with control). In contrast, angiotensin II increased SNGFR in ACE 2/2 mice to 9.1 ± 0.8 nl/min (P = 0.001 compared with control). Kidney weight correction did not alter the conclusion that angiotensin II significantly reduced SNGFR in WT and ACE 1/3 mice and increased it in ACE 2/2 mice (Fig. 5). Mean arterial blood pressure during the time of micropuncture increased with angiotensin II from 102.5 ± 3 to 109.6 ± 3.3 mmHg in WT (P = 0.02), from 68.5 ± 1.7 to 75 ± 2.4 mmHg in ACE 2/2 (P = 0.0004), and from 83 ± 2 to 90 ± 2 mmHg in ACE 1/3 mice (P < 0.0001). Blood pressure was significantly higher in WT than in ACE 2/2 mice (P < 0.01).

Proximal fractional reabsorption averaged 51.3 ± 3% in WT and 49 ± 2.3% in ACE 1/3 mice (not significant). In contrast, fractional reabsorption in ACE 2/2 mice was significantly higher, averaging 74.4 ± 3.5%. Rates of proximal tubular fluid reabsorption without and with correction for kidney weight are given in absolute terms (top) and following normalization for kidney weight (KW; bottom). Shown are significances for comparisons with WT mice (**P < 0.01 as tested by ANOVA), and nos. inside the bars are P values for comparisons between angiotensin II and control (paired t-test).
summarized in Fig. 6. While rates of absorption were significantly lower in both ACE 2/2 and ACE 1/3 than in WT mice when expressed in absolute terms, there were no differences in normalized reabsorption rates between the different genotypes. The relationship between SNGFR and proximal reabsorption showing identical slopes for WT and ACE 1/3 mice indicates that differences in absolute reabsorption reflect differences in kidney weight and not in intrinsic reabsorptive capacity (Fig. 7, top). In contrast, the glomerulotubular balance function was shifted upward in ACE 2/2 mice, reflecting the increased fractional reabsorption. Angiotensin II infusion increased fractional reabsorption to 60.4 ± 3.2% in WT (P = 0.05 compared with control) and decreased it in both ACE 2/2 and ACE 1/3 mice to 55.6 ± 2.3 and 40.8 ± 2.5%, respectively (P = 0.0004 and P = 0.02 compared with control). During the infusion of angiotensin II, the reabsorption rate increased significantly in ACE 2/2 mice compared with control, probably as a result of the markedly increased filtration rate. Reabsorption rates tended to decrease in WT mice, and they fell significantly in ACE 1/3 mice. The relationship between SNGFR and proximal reabsorption rate in individual tubules of WT, ACE 2/2, and ACE 1/3 mice suggests that the infusion of angiotensin II was associated in ACE 1/3 mice with a slight reduction of reabsorption at each level of SNGFR compared with either WT or ACE 2/2 mice (Fig. 7, bottom).

TGF. TGF was assessed as the reduction of PSF caused by a saturating increase in the loop of Henle perfusion rate (30 nl/min). The findings are summarized in Fig. 8. PSF fell from 38.8 ± 1.3 to 30.5 ± 1.4 mmHg in WT (n = 13; P < 0.0001) and from 31.8 ± 1.5 to 29.3 ± 1.3 mmHg in ACE 1/3 mice (n = 10; P = 0.02), whereas it did not significantly change in ACE 2/2 mice (28.3 ± 0.6 vs. 27.7 ± 0.6; n = 9; P = 0.07). The decrease in PSF by 8.3 ± 0.6 mmHg in WT mice was significantly greater than that of 0.7 ± 0.22 mmHg in ACE 2/2 (P < 0.001 compared with WT) and of 2.5 ± 0.9 mmHg in ACE 1/3 mice (P < 0.001 compared with WT). Mean arterial blood pressure in these studies was 107 ± 2.6 mmHg in WT, 70.1 ± 1.7 mmHg in ACE 2/2, and 85 ± 5.9 mmHg in ACE 1/3 mice.

**DISCUSSION**

The renin-angiotensin system influences numerous aspects of renal function related to the control of renal vascular resistance, net salt and water reabsorption, and the renal concentrating mechanism (15). While most of the actions of angiotensin II are mediated through the AT1 receptor, the peptide can also bind to the AT2 receptor, eliciting effects that widen the spectrum of its renal actions (3). One of the most vexing problems in understanding the consequences of changes in the activity of the renin-angiotensin system is related to the fact that angiotensin II can be generated systemically, with plasma renin being the rate-limiting step in most cases, but that...
Our results show that proximal tubular fluid reabsorption of these genetically altered mice was comparable to that observed in WT mice despite the essentially complete absence of tissue ACE. As is well known, proximal fluid reabsorption varied with SNGFR, but the relationship between SNGFR and proximal tubular reabsorption of fluid was not different between WT and ACE 1/3 mice, and it even shifted upward in ACE 2/2 mice as a reflection of an increase in fractional fluid reabsorption (Fig. 6, top). The mechanism for this increase in fractional fluid reabsorption in proximal tubules of ACE 2/2 mice was not explored in this study, but it is likely that it is related in some way to the reduced SNGFR. Our observations are unexpected in view of previous demonstrations that the intratubular administration of converting enzyme inhibitors caused a marked reduction in proximal fluid reabsorption (30) and that the same effect was seen with angiotensin II receptor blockers (31). These findings clearly show that an acute reduction in local angiotensin II formation and action can exert a profound inhibitory effect on fluid reabsorption. We assume therefore that the difference between these previous results and the present data lies in the chronicity of angiotensin II blockade. Chronic ACE deficiency is apparently associated with compensatory events that normalize fluid reabsorption along the proximal tubule. On the basis of these findings, one would not expect that chronic interference with local angiotensin II formation in the proximal tubule plays a major role in the maintenance of reductions in extracellular fluid volume. In fact, careful previous measurements have been unable to show measurable plasma volume contraction in either ACE 2/2 or ACE 1/3 mice (5, 7). A similar discrepancy between the effects of acute and chronic blockade of a transport-regulatory pathway has also been observed during inhibition of adenosine 1 receptors (A1R). While acute A1R blockade with CVT-124 caused a marked inhibition of proximal fluid reabsorption, proximal transport was unaltered in A1R-deficient mice (40, 41). Given the multifactorial nature of the regulation of proximal fluid transport, it is perhaps not unexpected that the chronic elimination of one regulatory input appears to be relatively inconsequential. It would be important to examine the related question of whether extracellular volume expansion can result from chronic overproduction of angiotensin II or other stimulatory transport regulators in the proximal tubule.

The infusion of angiotensin II at doses that caused a mild elevation of blood pressure did not significantly alter the rate of proximal fluid reabsorption remained unaltered in WT, ACE 2/2, and ACE 1/3 mice during control (top) and during angiotensin II infusion (bottom). Lines are linear regression lines calculated for the range of the data.
fluid reabsorption in WT mice, although there was a decrease in SNGFR. Thus angiotensin II infusion caused a mild stimulation of NaCl transport for a given SNGFR, shifting the reabsorption-SNGFR function curve slightly upward. Stimulation of proximal reabsorption during systemic infusion of angiotensin II has been described earlier (20). In contrast, fluid reabsorption fell in near proportion to SNGFR in angiotensin-infused ACE 1/3 mice. Since plasma angiotensin II levels in these animals have been found to be three times WT levels (7), it is conceivable that angiotensin II-dependent proximal reabsorption had reached its maximum before the angiotensin II infusion was started and that further increases in plasma angiotensin II were therefore not associated with further stimulation of transport (16). It is also conceivable that the elevated angiotensin II level caused downregulation of AT1 receptors throughout the organism.

In ACE 2/2 mice, angiotensin II infusion produced a marked increase in proximal fluid reabsorption (Fig. 6) that was paralleled by an increase in nephron as well as kidney GFR (Fig. 5) and may thus result from flow dependence of proximal fluid reabsorption, reflecting the well-known phenomenon of glomerulotubular balance. In addition, a direct effect of angiotensin II may contribute to transport stimulation. The cause for the increase in GFR is probably at least partly related to the increase in arterial blood pressure. However, angiotensin II elevated blood pressure to the same extent in WT and ACE 1/3 mice while lowering instead of increasing GFR. It is especially noteworthy that angiotensin II increased GFR in ACE 2/2 mice while at the same time doubling total renal vascular resistance (Fig. 3). Since a preglomerular vasoconstriction would result in a decrease in GFR, one is forced to assume that angiotensin II caused a predominantly postglomerular resistance increase in the ACE 2/2 mouse strain. Modeling suggests that an increase in postglomerular resistance causes an increase in GFR only at very low initial values of efferent resistance (24). Because of the low angiotensin II levels, low blood pressure, and the absence of TGF, it is likely that the glomerular arterioles of ACE 2/2 mice have a rather low resistance under ambient conditions (5). The notion that angiotensin II infused into the low-angiotensin II state of the ACE 2/2 mice may cause a predominantly efferent constriction is feasible in view of the evidence that efferent arterioles are more sensitive to angiotensin II than afferent arterioles when starting from zero angiotensin II, particularly at low pressures (42). The low initial filtration fraction (~7%) and its increase with angiotensin II (to ~12%) is consistent with this assumption, although it is in itself no proof for a predominantly efferent constriction (4).

The present studies show that mice without tissue ACE have a markedly reduced capacity to transform changes in luminal NaCl concentration into changes in glomerular capillary pressure. This TGF response deficit is reminiscent of earlier studies in AT1R and ACE knockout mice from our laboratory (34, 38). Furthermore, pharmacological inhibition of AT1R and ACE causes a significant reduction in TGF responsiveness (32). Conversely, the administration of angiotensin II by either systemic or peritubular infusion enhances TGF responses (21). The reduction in TGF responses in ACE 2/2 mice may be aided by the markedly reduced blood pressure since blood pressure has been shown earlier to be a determinant of the response magnitude (33). However, our observations in ACE 1/3 mice in which blood pressure was only marginally altered indicate that lack of angiotensin II availability contributes importantly to the attenuation of TGF responsiveness. Since plasma angiotensin II levels in ACE 1/3 mice have been found to be clearly higher than normal, the presence of angiotensin II in the systemic circulation appears to be insufficient to sustain normal TGF responses. It would appear rather that angiotensin II generation by tissue ACE is required to support this function. It is possible that tissue ACE in the vascular wall of afferent arterioles normally provides TGF-relevant angiotensin II. On the other hand, it is also conceivable that angiotensin II generated in the tubular lumen affects NaCl transport across macula densa cells and that this increase in transport enhances the basal sensitivity of the TGF system.

The present studies show that kidneys of ACE 2/2 mice are significantly hypoplastic compared with age-matched WT animals. The reasons for the reduced growth rate are unclear and need to be evaluated further. Reduced kidney weights compared with WT have also been reported in mice with combined AT1R and AT1BR deficiency, but in this case there was also a proportional difference in total body weight (27). Pharmacological blockade of angiotensin II receptors in neonatal rats also caused reduced somatic and renal growth (39). One of the functional consequences of the reduced kidney mass is that tissue-specific RBF in ACE 2/2 mice was not significantly different from WT, although absolute values were significantly lower in ACE 2/2 than in WT mice. In addition, our data show that the reduction in kidney GFR in ACE 2/2 mice compared with WT was more marked (~81%) than that in superficial nephron GFR (~58%). One explanation for this observation is that fewer nephrons contribute to total GFR in ACE 2/2 than WT mice. Calculation of the “effective” number of glomeruli from kidney and SNGFR yields values of ~24,000 in WT and ACE 1/3 but only ~9,000 in ACE 2/2 mice. While this calculation assumes equal rates of filtration in all nephrons of the kidneys and obviously only provides an estimate of the number of glomeruli, the difference appears to be clear and beyond all possible sources of error. A reduced glomerular number has also been reported to be associated with neonatal administration of losartan (39). Thus it appears that structural differences are partly responsible for the reduced renal function in ACE 2/2 mice.

In conclusion, our results show that the relationship between proximal tubule fluid reabsorption and filtration rate is comparable between WT mice and two strains of mice that lack tissue ACE activity. Thus the absence of angiotensin II generation in the proximal tubule does not appear to be associated with sustained inhibition of proximal fluid transport that could be directly responsible for volume depletion and reductions of arterial blood pressure. Generation of angiotensin II by tissue ACE contributes importantly to the magnitude of TGF responses.

ACKNOWLEDGMENTS

Present address of S. Hashimoto: Department of Medicine II, Hokkaido Univ., Sapporo, Hokkaido, Japan 060 8638.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grants DK-39777, DK-51445, and DK-55503 (K. E. Bernstein) and by intramural funds from the NIDDK. S. Hashimoto was the recipient of a Visiting Fellowship of the NIDDK.
REFERENCES


