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Kidney-specific enhancement of ANG II stimulates endogenous intrarenal angiotensinogen in gene-targeted mice

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Abstract

This study was performed in transgenic mice to test the hypothesis that the selective intrarenal overproduction of ANG II increases intrarenal mouse (m) angiotensinogen (AGT) expression. We used the following three groups: *1*) single transgenic mice (*group A*, $n = 14$) expressing human (h) AGT only in the kidney, *2*) double-transgenic mice (*group D*, $n = 13$) expressing human renin systemically in addition to hAGT only in the kidney, and *3*) wild-type (*group W*, $n = 12$) mice. Exogenous hAGT protein is inactive in *group A* because endogenous mouse renin cannot cleave hAGT to ANG I because of a high species specificity. All mice were monitored from 12 to 18 wk of age. Systolic blood pressure progressively increased from 116 ± 5 mmHg (12 wk) to 140 ± 7 (18 wk) in *group D*. This increase was not observed in *groups A* or *W*. Intrarenal hAGT levels were similar in *groups A* and *D*; however, hAGT was not detectable in kidneys of *group W*. Kidney ANG II levels were increased in *group D* (216 ± 43 fmol/g) compared with *groups A* (117 ± 16) and *W* (118 ± 17). However, plasma ANG II concentrations were similar among the three groups. Endogenous renal mAGT mRNA was increased significantly in *group D* (1.46 ± 0.19, ratio) compared with *groups A* (0.97 ± 0.12) and *W* (1.00 ± 0.08). Endogenous renal mAGT protein was also significantly increased in *group D* compared with *groups A* and *W*. Interstitial collagen-positive area, interstitial macrophage/monocyte infiltration, and afferent arteriolar wall thickness were increased significantly in *group D* compared with *groups A* and *W*. These data indicate for the first time that the selective stimulation of intrarenal production of ANG II from hAGT augments endogenous intrarenal mAGT mRNA and protein expression.

Keywords

hypertension; transgenic mouse; angiotensin II; renal injury

Recent attention has been focused on the existence of unique renin-angiotensin systems (RAS) in various tissues (10). Emerging evidence has demonstrated the importance of the tissue RAS in the brain (1), heart (7), adrenal glands (34), vasculature (5,13,15,36), and kidneys (37). The RAS has been acknowledged as an endocrine, paracrine, autocrine, and intracrine system and thus it has been difficult to delineate the quantitative contributions of systemically delivered vs. locally formed ANG peptides to the levels existing in any given tissue (37). In this regard, the kidneys are unique in terms of the tissue concentrations of ANG II, which are much greater than can be explained by the concentrations delivered by

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the arterial blood flow (19). There is substantial evidence that the major fraction of ANG II present in renal tissues is generated locally from angiotensinogen (AGT) delivered to the kidney as well as from AGT locally produced by proximal tubule cells (18). Renin secreted by the juxtaglomerular apparatus cells in the renal interstitium and vascular compartment also provides a pathway for the local generation of ANG I (35). ANG-converting enzyme is abundant in the rat kidney and is present in proximal and distal tubules, the collecting ducts, and renal endothelial cells (3). ANG I delivered to the kidney can also be converted to ANG II (31). Therefore, all of the components necessary to generate intrarenal ANG II are present along the nephron (29).

Previous studies performed primarily in rats have shown that systemic ANG II infusions increase intrarenal ANG II levels, partially because of concomitant increases in proximal tubular AGT mRNA and protein further augmenting intrarenal ANG II levels, leading to progressive development of hypertension and renal injury in ANG II-infused rats (23–25,27,30). However, it has not been established if selective increases in intrarenal ANG II can be responsible for the stimulation of intrarenal AGT along with the development of progressive hypertension and/or renal injury. With the use of a transgenic mouse model in which human (h) AGT is expressed only in the kidney (6,8,9), experiments were performed to determine if selective renal overproduction of ANG II elicited by stimulating hAGT present only in the kidney in the presence of human renin (hR) will cause increases in endogenous mouse (m) AGT mRNA and protein expression in kidneys. The primary objective of this study was to determine if intrarenally produced ANG II elicited by activating the transgenes would stimulate endogenous mAGT. Because the hAGT is localized only in the kidney, the double-transgenic mice also harboring the *hR* gene will have an increased intrarenal ANG II generated from hAGT. Thus, by using this model, it was possible to determine if such localized increases in ANG II resulting from activating hAGT would, in turn, lead to augmentation of intrarenal production of endogenous mAGT, which in turn could lead to further increases in intrarenal ANG II levels. Secondarily, we extended the study to evaluate if the hypertension and intrarenal ANG II levels elicited in this manner were also associated with the early indexes of proliferation and/or inflammatory responses in the kidneys as has been observed in rats infused with exogenous ANG II (23–25,27,30).

MATERIALS AND METHODS

Preparation of animals

The experimental protocol was approved by the Animal Care and Use Committees of Tulane University and University of Iowa. We used the following three groups of male mice: *1*) single transgenic mice (*group A*, $n = 14$) expressing hAGT only in the kidney regulated by kidney-specific androgen-regulated protein promoter, *2*) double-transgenic mice (*group D*, $n = 13$) expressing hR systemically in addition to hAGT only in the kidney, and *3*) wild-type (*group W*, $n = 12$) mice of genetic background C57BL/6J. These mice have been characterized in previous studies (6,8,9). Exogenous hAGT protein is inactive in *group A* mice because endogenous mouse renin cannot cleave hAGT to ANG I because of high species specificity (12,16). As previously described (11,42), mice with only systemic overexpression of hR do not show an increase in blood pressure (BP) because of a high species specificity (12,16). hR does not cleave ANG I from mAGT and thus we did not include a group with only systemic overexpression of hR in this study. In the colonies used for this study, systolic BP at 18 wk of age in mice with only systemic overexpression of hR was similar to that of *group W* mice (data not shown). All mice were monitored from 12 to 18 wk of age with free access to a regular diet and water. Systolic BP was measured in conscious mice using tail-cuff plethysmography (BP-2000; Visitech) one time per week as previously described (23–25,27,30).

Sample collection

Blood, kidney, and liver samples were harvested at 18 wk of age. After decapitation, trunk blood was collected into chilled tubes containing EDTA (5 mmol/1), enalaprilat (20 μmol/l), pepstatin A (10 μmol/l), and 1,10-phenanthroline (1.25 mmol/1). Plasma was separated and stored at −20°C until assayed for plasma ANG H as previously described (23–25,27,30). Immediately after removal, one kidney was homogenized in cold methanol, and renal ANG II was measured as previously described (23–25,27,30). The contralateral kidneys were separated into three pieces and immersed in RNAlater (Ambion) for total RNA extraction, immersed in zinc-saturated formalin (Anatech) for tissue fixation, and snap-frozen in liquid nitrogen for protein extraction. A small piece of liver was also collected in RNAlater for total RNA extraction.

Quantitative real-time RT-PCR

Total RNA extraction from mouse kidney and liver and quantitative real-time RT-PCR for exogenous hAGT mRNA and endogenous mAGT mRNA were performed as previously described (28,39). Data of quantitative real-time RT-PCR were normalized by glyceraldehydes-3-phosphate dehydrogenase (GAPDH) mRNA expression. The information of sequences was as follows: exogenous hAGT mRNA, forward primer, 5′-CAG AAC TGG ATG TTG CTG CT-3′, reverse primer, 5′-GTT GTC CAC CCA GAA CTC CT -3′, probe, 5′-/56-FAM/ GCT TTC AAC ACC TAC GTC CAC TTC CAA /3BHQ-1/-3′; endogenous mAGT mRNA, forward primer, 5′-AAA GCA GGA GAG GAG GAA CA-3′, reverse primer, 5′-TGA GTC CTG CTC GTA GAT GG-3′, probe, 5′-/56-FAM/ ACT GGA TGT GAC CCT GAG CAG CC /3BHQ-1/-3′; GAPDH mRNA, forward primer, 5′-CAG AAC ATC ATC CCT GCA TC-3′, reverse primer, 5′-CTG CTT CAC CAC CTT CTT GA -3′, probe, 5′-/5-HEX/ CCT GGA GAA ACC TGC CAA GTA TGA TGA /3BHQ-2/-3′.

Development of antibodies

For this study, two lines of antibodies were newly raised using a custom service (Zymed Laboratories). Human-specific AGT antibodies were raised in chickens against a synthetic peptide (C)TELNLQKLSNDRIR, that is highly specific for humans. Purification of IgY-rich fraction was also done by Zymed Laboratories. Rodent-specific AGT antibodies were raised in rabbits against a synthetic peptide, (C)AGEEEQPTESAQQPGSPE, that is highly specific for rodents. Affinity purification was also done by Zymed Laboratories.

Western blot analysis

Protein extraction from mouse kidney and Western blot analysis for AGT and β-actin were performed as previously described (23–25,27,30). Data of Western blot analysis for endogenous mAGT protein levels were normalized by β-actin protein levels.

Evaluation of renal injury

With the use of zinc-saturated formalin-fixed paraffin-embedded renal sections, the early indexes of proliferation or inflammatory responses in mice were determined by the following three parameters: *1*) interstitial collagen-positive area, *2*) interstitial macrophage/monocyte infiltration, and *3*) afferent arteriolar hypertrophy.

1. The extent of interstitial collagen-positive area was quantitatively evaluated by automatic image analysis of the area occupied by interstitial tissue staining positively for collagen in PicroSirius Red-stained sections (Mass Histology) as previously described (14,26,38). The fraction of renal cortex occupied by interstitial tissue was performed using the Image-Pro plus software (Media Cybernetics). For each microscopic field, the collagen-positive area (pink) was

automatically calculated by the software, and this affected area was in turn divided by the total area of the microscopic field. Twenty consecutive microscopic fields were examined for each mouse, and the averaged percentages of the collagen-positive lesions were obtained for each mouse.

2. The number of macrophages/monocytes was examined by immunohistochemistry using a commercially available antibody against CD68 (Serotec) as previously described (20,26,38). Immunohistochemistry was performed by a robotic system (Autostainer; Dako) as previously described (26,38) and counterstained with hematoxylin-eosin. Twenty consecutive microscopic fields were examined for each mouse, and CD68-positive cells (brown) were counted in interstitium in each microscopic field. The averaged numbers of macrophages/monocytes in interstitium were then obtained for each mouse.

3. The proliferation of afferent arteriolar wall was evaluated by immunohistochemistry using a commercially available antibody against α-smooth muscle isoform of actin (Sigma) as previously described (2,26,38). Immunohistochemistry was performed by a robotic system as previously described (26,38). Because α-smooth muscle actin was expressed on both afferent and efferent arterioles, elastin was used, which stains only preglomerular vessels, to identify afferent arterioles. The thickness of the wall of afferent arterioles was then measured with microscopy (brown vessels besides a glomerulus in the center of the microscopic field). Twenty afferent arteriolar walls were examined for each mouse, and the averaged thickness of the wall of afferent arterioles was determined for each mouse.

The histological analyses were performed by an outsourcing company (Mass Histology) or a robotic system with an automatic image analysis software (Media Cybernetics) in a blind manner to avoid any biases.

Additional animal protocol

An additional cohort of three groups (*groups A, D,* and *W*) of male mice (18 wk of age, $n = 4$ in each) was prepared, and 24-h urine samples were collected using metabolic cages (Hatteras). Plasma samples were obtained from mice as described above. We have recently developed a sensitive and accurate method to measure hAGT using sandwich-type enzyme-linked immunosorbent assay (ELISA) in which a combination of two lines of human-specific AGT antibodies are used. Levels of hAGT in urine and in plasma from mice were measured by this human-specific AGT ELISA as described recently (21).

Statistical analysis

Statistical analysis was performed using a one-way factorial ANOVA with post hoc Scheffé's *F* test. All data are presented as means ± SE. $P < 0.05$ was considered significant.

RESULTS

Systolic BP

Systolic BPs (Fig. *1 A*) were similar at 12 wk of age among the three groups. Systolic BP progressively increased from 116 ± 5 mmHg (12 wk) to 140 ± 7 (18 wk) in double-transgenic mice (*group D*) expressing hR systemically in addition to hAGT only in the kidney during this period. This increase was not observed in single-transgenic mice (*group A*, 117 ± 2 at 18 wk) expressing hAGT only in the kidney or wild-type mice (*group W*, 116 ± 2 at l8 wk).

Exogenous hAGT mRNA

Figure 1*B* demonstrates a representative amplification plot of the real-time RT-PCR for exogenous hAGT mRNA. Kidney RNA samples from *group A* and *group D* mice demonstrated an equivalent amplification. Kidney RNA samples from *group W* mice did not show any amplification. Moreover, liver samples from either *group A, group D,* or *group W* mice did not exhibit any amplification. These data clearly indicate that the exogenous hAGT mRNA was expressed in the kidneys of *group A* and *group D* mice and not in the liver, showing that hAGT does not simply colocalize with mAGT.

Exogenous hAGT protein

Figure 1*C* demonstrates a representative Western blot analysis for exogenous hAGT protein. The antibody specific for hAGT recognized AGT in human kidney and plasma but not in rat kidney. We previously reported that Western blot analysis for rat AGT demonstrates double bands in plasma but a single band in kidney because of the different magnitude of glycosylation (24). This interpretation may account for the double band in human plasma and the single band in human kidney. With the use of this human-specific AGT antibody, kidney protein samples from *group A, D,* and *W* mice were evaluated. As depicted in Fig. 1 *C,* this human-specific AGT antibody demonstrated AGT protein in *group A* and *D* mice but not in *group W* mice. These data clearly indicate that the exogenous hAGT protein was expressed in the kidneys of *group A* and *D* mice.

Plasma and kidney ANG II levels

As demonstrated in Fig. 1*D,* plasma ANG II concentrations were similar among the three groups. However, kidney ANG II levels were increased significantly in *group D* mice (216 ± 43 fmol/g) compared with *group A* (117 ± 16) and *group W* (118 ± 17) mice, as depicted in Fig. 1*E.* Thus selective stimulation of intrarenal hAGT in *group D* mice leads to increases in ANG II levels that are restricted to the kidney and do not lead to increases in plasma ANG II levels, indicating no perceptible release of intrarenal ANG II systemically.

Endogenous mAGT mRNA

Figure 2*A* demonstrates that endogenous mAGT mRNA levels in the kidney were significantly increased in *group D* mice (1.46 ± 0.19, relative ratio) compared with *group A* (0.97 ± 0.12) and *group W* (1.00 ± 0.08) mice. This augmentation was limited to the kidney, and endogenous mAGT mRNA levels in the liver were not altered among the three groups as described in Fig. 2*B.*

Endogenous mAGT protein

Figure 2*C* demonstrates a representative Western blot analysis for endogenous mAGT protein. The antibody specific for rodent AGT recognizes AGT in mouse kidney and rat kidney but not in human kidney. With the use of this rodent-specific AGT antibody, kidney protein samples from *group A, D,* and *W* mice were evaluated. As depicted in Fig. 2*D,* (3-actin protein levels were similar among the groups. However, as demonstrated in Fig. 2*E,* endogenous mAGT protein in the kidney was increased significantly in *group D* (1.49 ± 0.02, relative ratio) compared with *group A* (1.03 ± 0.04) and *group W* (1.00 ± 0.03) mice.

Interstitial collagen-positive area

The interstitial collagen-positive area was stained by PicroSirius Red using zinc-saturated formalin-fixed paraffin-embedded kidney samples from *group W* (Supplemental Fig. 1A), *group A* (Supplemental Fig. 1B), and *group D* (Supplemental Fig. 1C) mice. (Supplemental material for this article can be found at the *American Journal of Physiology: Renal Physiology* web site.) The collagen-positive area is stained in pink. Analysis using a

computer-aided semiautomatic quantification system demonstrated that the interstitial collagen-positive area was increased significantly in *group D* mice (0.52 ± 0.06%) compared with *group A* (0.36 ± 0.05) and *group W* (0.34 ± 0.03) mice as depicted in Supplemental Fig. 1D.

Interstitial macrophage/monocyte infiltration

The interstitial macrophage/monocyte infiltration was evaluated by CD68-positive cell number, which is a surface marker for macro-phages and monocytes, using zinc-saturated formalin-fixed paraffin-embedded kidney samples from *group W* (Fig. 3*A*), *group A* (Fig. 3*B*), and *group D* (Fig. 3*C*) mice. CD68-positive cells are stained in brown. CD68-positive cell numbers were increased significantly in *group D* mice (46 ± 5 cells/mm^2) compared with *group A* (20 ± 3) and *group W* (19 ± 2) mice, as demonstrated in Fig. 3*D*.

Afferent arteriolar hypertrophy

The thickness of afferent arteriolar wall was visualized by immunohistochemistry of α-smooth muscle isoform of actin and elastin stain using zinc-saturated formalin-fixed paraffin-embedded kidney samples from *group W* (Fig. 4*A*), *group A* (Fig. 4*B*), and *group D* (Fig. 4*C* mice. Afferent arteriolar walls are stained in brown and purple. The thickness of afferent arteriolar wall was increased significantly in *group D* mice (3.31 ± 0.41 μm) compared with *group A* (2.21 ± 0.12) and *group W* (2.16 ± 0.11) mice, as demonstrated in Fig. 4*D*.

Levels of hAGT in urine and in plasma from mice

Using a human-specific AGT ELISA system, we measured 12 urinary samples from *group W* mice (*n* = 4), *group A* mice (*n* = 4), and *group D* mice (*n* = 4) and 12 plasma samples from *group W* mice (*n* = 4), *group A* mice (*n* = 4), and *group D* mice (*n* = 4). Our ELISA system clearly showed that urinary samples from *group A* and *group D* mice have detectable levels of hAGT (7.4 ± 2.1 and 11.5 ± 2.2 ng/ml, respectively). However, urinary samples from *group W* mice do not have detectable levels of hAGT. Moreover, we were not able to detect any hAGT in plasma samples from either mouse. These data indicate that leak of exogenous hAGT in the plasma in *group A* mice or *group D* mice is negligible.

DISCUSSION

With the use of a transgenic mouse model in which hAGT is expressed only in the kidney (6,8,9), the present data demonstrate that selective renal overproduction of ANG II elicited by stimulating hAGT present only in the kidney in the presence of hR increases endogenous mAGT mRNA and protein expression in kidneys, leading to slowly progressive hypertension and the associated proliferative and inflammatory early responses suggesting initiation of renal injury. In preliminary studies, we confirmed that systolic BP in *group D* mice was not increased before 12 wk of age. Moreover, systolic BP in *group D* mice increased during the subsequent 6 wk and did not increase further after 18 wk of age. The *group D* mice remain hypertensive after this age (data not shown), but we chose to study them at 18 wk to investigate primarily the early events. We did not measure plasma testosterone levels of these mice because of the lack of the plasma sample volumes. However, the temporal profile of systolic BP suggests that sexual maturation starts at 12 wk of age and is fully functional by 18 wk of age in these mice.

For this study, two lines of antibodies against AGT were developed. One is specific for hAGT; the other is specific for rodent AGT. The antibody specific for hAGT recognized AGT in human kidney and plasma but not in rat kidney or *group W* mice kidney, as shown in Fig. 1*C*. In contrast, the antibody specific for rodent AGT recognized AGT in mouse

kidney and rat kidney but not in human kidney, as demonstrated in Fig. 2*C*. These data clearly indicate that the two antibodies were specific for hAGT and specific for rodent AGT.

Previous studies have shown that ANG II-infused rats have increases in renal AGT mRNA (24,41) and protein (23) contributing to the elevated intrarenal ANG II levels. Transgenic mice expressing hAGT only in the kidney develop hypertension when a source of hR is available (6,8,9). The effects of the ANG II formed from hAGT could be amplified by the local augmentation mechanism by which mAGT was stimulated. Several in vitro studies have demonstrated ANG II-induced augmentation of AGT mRNA expression. Klett et al. (22) presented evidence that ANG II enhances hepatic AGT synthesis by inhibiting degradation of AGT mRNA in hepatocytes. Li and Brasier (32) suggested that activation of the *AGT* gene by ANG II is mediated by the nuclear factor-κB p65 transcription factor in hepatocytes. Tamura et al. (43) showed that ANG II activates transcription of the *AGT* gene exclusively via the ANG II type 1 (AT_1) receptor pathway in cardiac myocytes. Mascareno et al. (33) showed that activation of the AGT promoter by ANG II depends on the signal transducer and activator of transcription protein signal pathway in cardiac myocytes. Although less is known about the amplification mechanisms in renal tissues, Ingelfinger et al. (17) demonstrated an enhanced AGT mRNA expression by ANG II in an immortalized proximal tubular cell line. These findings support the concept that selective increases in intrarenal ANG II stimulate its precursor, AGT, in the kidney, thus leading to the enhanced intrarenal ANG II formation and the development of progressive hypertension and renal injury in this study. The present study provides direct evidence demonstrating that mAGT mRNA and protein are indeed augmented following stimulation of local ANG II formation from hAGT. Further studies will be needed to determine the quantitative roles of hAGT vs. mAGT to the augmented intrarenal ANG II levels, and the role of activation of mAGT is essential in the subsequent development of the progressive hypertension and the associated proliferative and inflammatory responses initiating renal injury.

The importance of the intrarenal RAS in the development of hypertension and renal injury is also reported in a recent study using a different transgenic mouse model. Sachetelli et al. (40) generated transgenic mice in which kidney-specific overexpression of rat AGT significantly increased BP and initiated renal injury. They also report that treatment with an AT_1 receptor antagonist or with an angiotensin-converting enzyme inhibitor reversed these abnormalities in transgenic animals. Our results extend these findings and provide novel information by showing that locally generated ANG II leads to further stimulation of endogenous mAGT. The crucial role of intrarenal ANG II in the development of hypertension is also reported in a recent study by Crowley et al. (4) using a kidney cross-transplantation model. They clearly illustrated in the systemic AT_{1A} receptor knockout mice that the presence of AT_{1A} receptors only in the kidney is sufficient to recapitulate the phenotype of hypertension with ANG II infusion. Conversely, in the renal AT_{1A} receptor knockout group, the absence of AT_{1A} receptors from the kidney alone is sufficient to protect these mice from ANG II-dependent hypertension, despite the expression of AT_{1A} receptors in a number of other key areas that potentially impact BP homeostasis. Although actions of the RAS in a variety of target organs have the potential to promote high BP and tissue injury, they concluded that ANG II causes hypertension primarily through effects on AT_1 receptors in the kidney. The intrarenal ANG II augmentation mechanism demonstrated in the present study helps to explain the potent role of intrarenal ANG II in the development of hypertension.

The observed effects could be directly related to increased intrarenal ANG II levels or to the associated increased BP effects of ANG II. Although not specifically addressed in the present study, we have addressed this issue in a recent paper (28). Spontaneously hypertensive rats (SHR) showed the enhanced intrarenal ANG II and hypertension and renal

injury at 14 wk of age compared with SHR at 7 wk of age. Two groups of SHR received either an AT_1 receptor blocker (olmesartan, 5 mg/day) or triple therapy (hydralazine, 7.5 mg/day; reserpine, 0.15 mg/day; hydrochlorothiazide, 3 mg/day) during *weeks 7–14*. Olmesartan treatment and the triple-therapy treatment both prevented hypertension. Although olmesartan treatment prevented the augmented intrarenal ANG II and the development of renal injury, the triple-therapy treatment failed to prevent the augmented intrarenal ANG II or the development of renal injury. These results indicate that the development of renal injury in SHR at 14 wk of age was more related to intrarenal ANG II than BP.

It is not clear in this study why an increase in BP failed to downregulate the endogenous circulating ANG II levels, although this may have been balanced by increased ANG II release from the kidneys. Liver mAGT mRNA levels tended to decrease in the *group D* mice compared with other groups in this study; however, the difference is not statistically significant. The increase in BP in the *group D* mice (24 mmHg) may fail to downregulate endogenous ANG II because of the local amplification mechanisms, as previously shown in ANG II-infused rats. From studies in rats, it has become clear that, although the hypertension clearly downregulates endogenous juxtaglomerular apparatus renin mRNA and protein, the intrarenal ANG II levels actually increase in a manner apparently independent of juxtaglomerular apparatus renin. This augmentation is the result of several factors, including the presence of renin in proximal and distal nephron segments (39).

In conclusion, these data indicate for the first time that the selective intrarenal overproduction of ANG II stimulates endogenous intrarenal mAGT mRNA and protein expression, which may contribute to the slowly progressive hypertension and the associated initiation of renal injury in the gene-targeted mice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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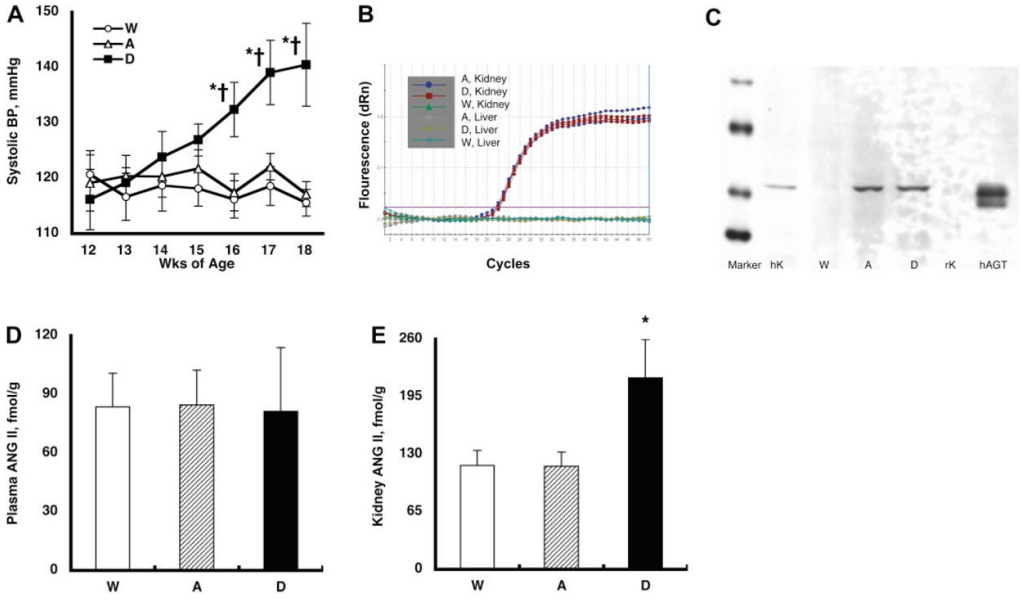

Fig. 1.

A: temporal profile of systolic blood pressure (BP). Systolic BP was similar at 12 wk of age among the three groups. However, systolic BP progressively increased from 116 ± 5(12 wk) to 140 ± 7 (18 wk) mmHg in double-transgenic mice (*group D*) expressing human renin (hR) systemically in addition to human (h) angiotensinogen (AGT) only in the kidney during this period. This increase was not observed in single-transgenic mice (*group A*, 117 ± 2 at 18 wk) expressing hAGT only in the kidney or wild type mice (*group W*, 116 ± 2 at 18 wk). $P < 0.05$ compared with the corresponding *group W* mice at that time period (*) and compared with the corresponding group at 12 wk of age (†). *B*: representative amplification plot of the real-time RT-PCR for exogenous hAGT mRNA. Kidney RNA samples from *group A* and *group D* mice demonstrated a nice and equivalent amplification. Kidney RNA samples from *group W* mice did not show any amplification. Moreover, liver samples from either *group A*, *group D*, or *group W* mice did not exhibit any amplification. These data clearly indicate that the exogenous hAGT mRNA were expressed only in the kidneys of *group A* and *D* mice. *C:* representative Western blot analysis for exogenous hAGT protein. The developed antibody specific for hAGT recognized AGT in human kidney (hK, 10 μg of protein) and plasma (50 ng; Calbiochem) but not in rat kidney (rK, 10 μg of protein). With the use of this human-specific AGT antibody, kidney protein samples (10 μg of protein) from *group A*, *D*, and *W* mice were evaluated. This human-specific AGT antibody demonstrated AGT protein in *group A* and *D* mice but not in *group W* mice. These data clearly indicate that the exogenous hAGT protein was expressed only in the kidneys of *group A* and *D* mice. *D*: plasma ANG II concentrations. Plasma ANG II levels were similar among the 3 groups. *E:* kidney ANG II contents. Kidney ANG II levels were increased significantly in *group D* mice (216 ± 43 fmol/g) compared with *group A* (117 ± 16) and *group W* (118 ± 17) mice. *$P < 0.05$ compared with the *group W* mice.

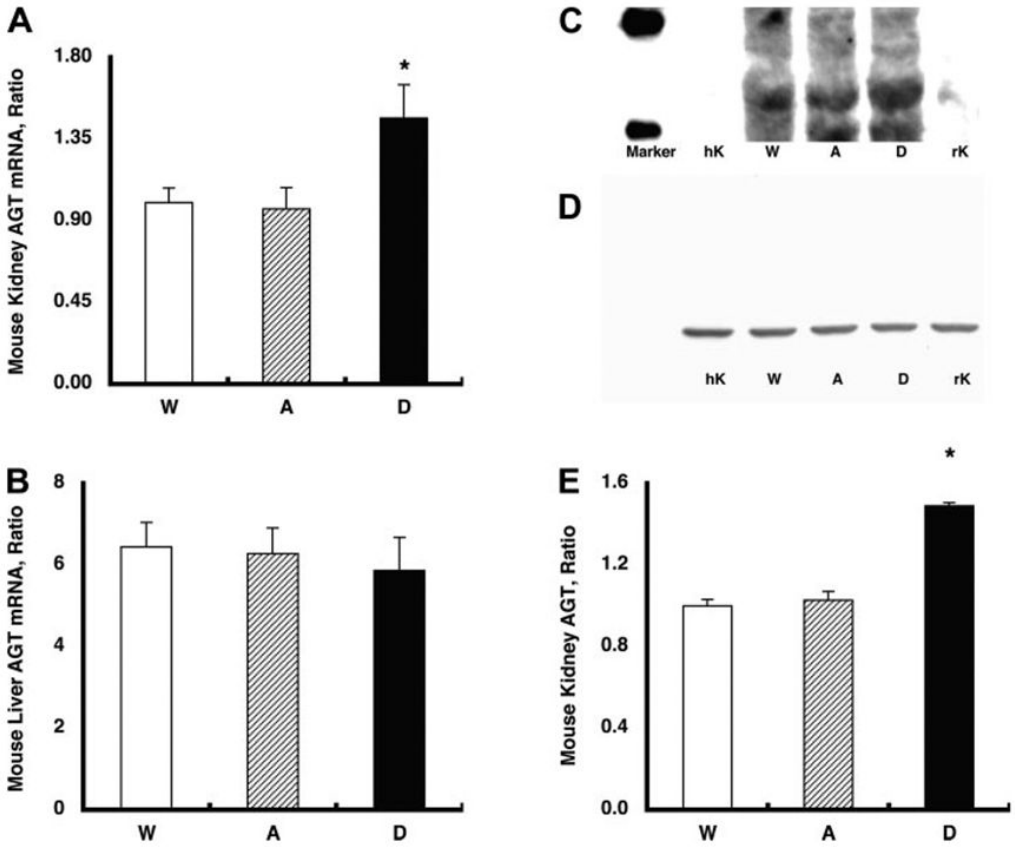

Fig. 2.

A: endogenous mouse (m) AGT mRNA levels in the kidney. Endogenous mAGT mRNA levels in the kidney were increased significantly in *group D* mice (1.46 ± 0.19, relative ratio) compared with *group A* (0.97 ± 0.12) and *group W* (1.00 ± 0.08) mice. **P* < 0.05 compared with the *group W* mice. *B*: endogenous mAGT mRNA levels in the liver. The augmented endogenous mAGT levels were limited in the kidney because endogenous mAGT mRNA levels in the liver were not altered among the three groups. *C*: representative Western blot analysis for endogenous mAGT protein. The developed antibody specific for rodent AGT recognized AGT in mouse kidney (10 μg of protein) and rat kidney (10 μg of protein) but not in human kidney (10 μg of protein). With the use of this rodent-specific AGT antibody, kidney protein samples from *group A, D,* and *W* mice were evaluated. *D*: representative Western blot analysis for β-actin protein showing that β-actin protein levels were similar among the groups. *E:* densitometric analysis demonstrated that endogenous mAGT protein in the kidney was also increased significantly in *group D* (1.49 ± 0.02, relative ratio) compared with *group A* (1.03 ± 0.04) and *group W* (1.00 ± 0.03) mice. **P* < 0.05 compared with the *group W* mice.

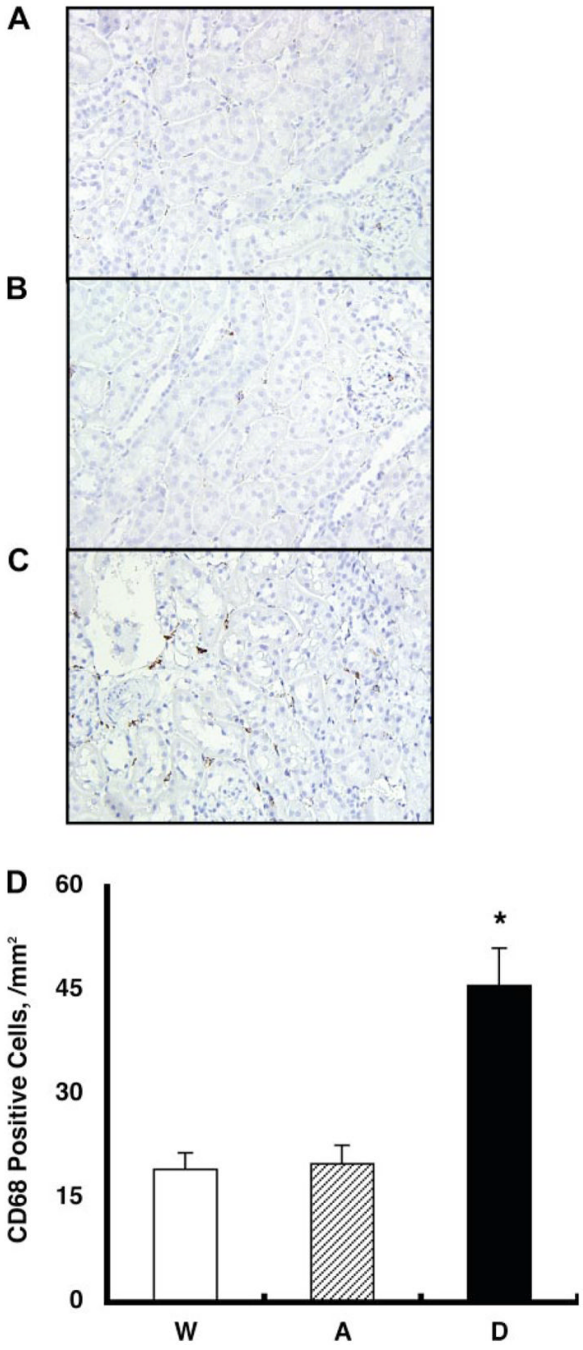

Fig. 3.

Interstitial macrophage/monocyte infiltration was evaluated by CD68-positive cell number, which is a surface marker for macrophages and monocytes, using zinc-saturated formalin-fixed paraffin-embedded kidney samples from *group W* (*A*), *group A* (*B*), and *group D* (*C*) mice. CD68-positive cells are stained in brown. *D:* CD68-positive cell numbers were increased significantly in *group D* mice (46 ± 5 cells/mm^2) compared with *group A* (20 ± 3) and *group W* (19 ± 2) mice. **P* < 0.05 compared with the *group W* mice.

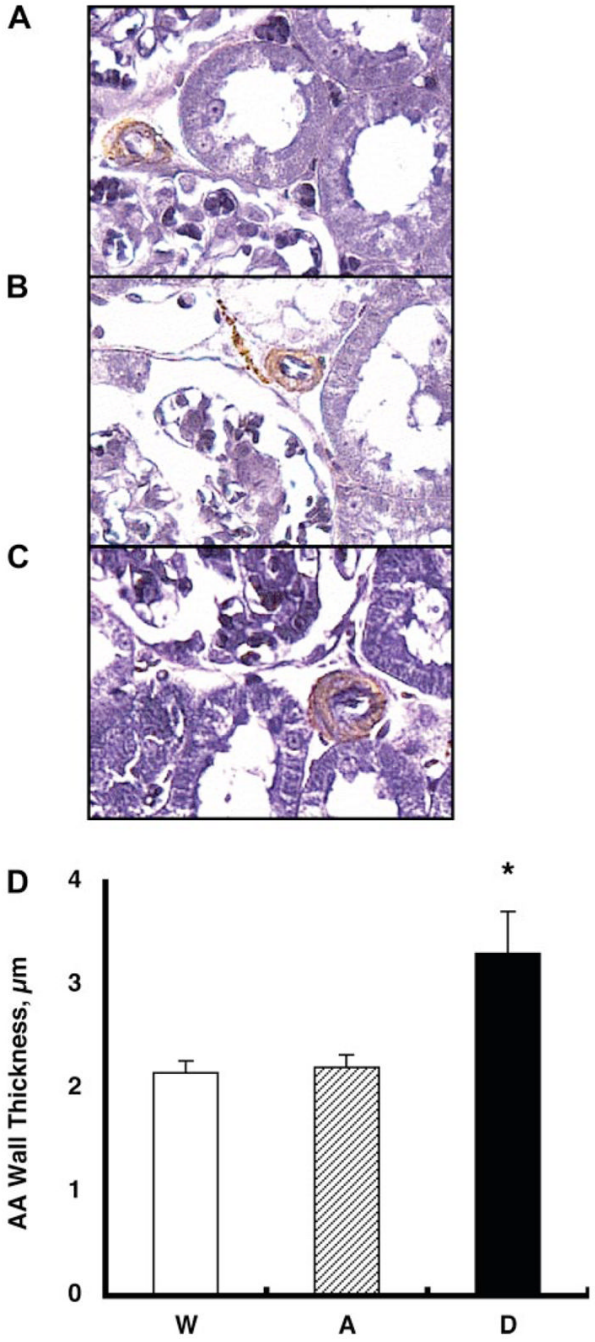

Fig. 4.

The thickness of afferent arteriolar (AA) wall was visualized by immunohistochemistry of α-smooth muscle isoform of actin and elastin stain using zinc-saturated formalin-fixed paraffin-embedded kidney samples from *group W* (*A*), *group A* (*B*), and *group D* (*C*) mice. Afferent arteriolar walls are stained in brown and purple. *D*: the thickness of afferent arteriolar wall was increased significantly in *group D* mice (3.31 ± 0.41 μm) compared with *group A* (2.21 ± 0.12) and *group W* (2.16 ± 0.11) mice. **P* < 0.05 compared with *group W* mice.