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H. Kobori, A. Katsurada, K. Miyata, N. Ohashi, R. Satou, T. Saito, Y. Hagiwara, K. Miyashita and L. G. Navar

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Novel mechanism of hypertension revealed by cell-specific targeting of human angiotensinogen in transgenic mice

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Davissou, Robin L., Yueming Ding, David E. Stec, James F. Catterall, and Curt D. Sigmund. Novel mechanism of hypertension revealed by cell-specific targeting of human angiotensinogen in transgenic mice. *Physiol. Genomics* 1: 3–9, 1999.—We tested the hypothesis that the tissue-specific intrarenal renin-angiotensin system (RAS) can participate in the regulation of blood pressure independently of its endocrine counterpart, by generating two transgenic models that differ in their tissue-specific expression of human angiotensinogen (AGT). Human AGT expression was driven by its endogenous promoter in the systemic model and by the kidney androgen-regulated protein promoter in the kidney-specific model. Using molecular, biochemical, and physiological measurements, we demonstrate that human AGT mRNA and protein are restricted to the kidney in the kidney-specific model. Plasma ANG II was elevated in the systemic model but not in the kidney-specific model. Nevertheless, blood pressure was markedly elevated in both the systemic and kidney-specific transgenic mice. Acute administration of the selective ANG II AT-1 receptor antagonist losartan lowered blood pressure in the systemic model but not in the kidney-specific model. These results provide evidence for the potential importance of the intrarenal RAS in blood pressure regulation by showing that expression of AGT specifically in the kidney leads to chronic hypertension independently of the endocrine RAS.

transgenic mice; blood pressure regulation; renal function; angiotensin II

HYPERTENSION is a major risk factor for cardiovascular morbidity and mortality and exhibits a complex etiology involving both genetic and environmental factors. Although numerous studies have implicated the renin-angiotensin system (RAS) in the development of hypertension, the precise mechanism remains unclear. The RAS has long been known to be an important mediator of systemic blood pressure and electrolyte balance through the systemic and intrarenal actions of angiotensin II (ANG II). Physiological, biochemical, and molecular biological studies have provided convincing evidence for multiple pathways of ANG II production. The classical or endocrine pathway involves the blood-borne generation of ANG II by the catalytic processing of angiotensinogen (AGT) released from the liver by renin in the systemic circulation. In addition to the endocrine RAS, local synthesis of ANG II is thought to occur in tissues that contain a local source of AGT. AGT mRNA

has been reported in proximal tubule cells, and the concentration of ANG II in proximal tubular fluid is higher than can be accounted for by filtration of circulating ANG II (13, 22, 31). These data suggest that ANG II can be synthesized within the kidney by the processing of AGT released from proximal tubule epithelial cells. Recent data suggest that intrarenally derived ANG II may be a potent regulator of systemic blood pressure (BP) (4, 22) and renal function (17, 29). This is supported by intrarenal production of components necessary for synthesis and action of ANG II, induction of the intrarenal RAS under certain experimental and disease conditions, and differential regulation of intrarenal and plasma-borne ANG II (1, 13, 25). However, because circulating ANG II can access the kidney through the renal circulation, the importance of intrarenally generated ANG II remains unclear. Indeed, it has been difficult to experimentally distinguish between the endocrine and intrarenal RAS because of problems associated with the specificity and delivery of pharmacological inhibitors that would prevent them from preferentially inhibiting one system over another.

To assess the importance of intrarenal ANG II, we designed a transgene to limit its normal widespread tissue distribution to a single organ and cell type. This was accomplished by placing the coding region of the human AGT gene downstream of a kidney-specific and proximal tubule-specific promoter (19). Transgenic mice were generated that exhibit androgen-inducible expression of human AGT specifically targeted to proximal convoluted tubule cells of the kidney (6). There are several attractive features of this transgene that provided us with a tool to differentiate between blood-borne and intrarenal ANG II. First, because proximal tubule cells are the normal site of endogenous AGT synthesis in this organ, we were able to restrict the cell-specific expression of AGT without altering its normal pattern of expression within the kidney (13, 19). Second, because the promoter is not active in the liver, the source of circulating human AGT is effectively eliminated. Third, because the promoter is regulated by androgen, its expression in females could be easily modulated by administration of a hormonal implant.

Using these mice, we tested the hypothesis that intrarenal production of ANG II, derived from AGT synthesized in proximal tubule cells, can be an important mediator of BP regulation in some experimental circumstances and, if overexpressed in transgenic mice, may lead to systemic hypertension independently of circulating ANG II. The methods described herein may

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provide a new paradigm to experimentally dissect other complex physiological systems.

METHODS

Transgenic mice. Single-transgenic mice expressing human renin systemically, human AGT systemically, and human AGT intrarenally and double-transgenic mice expressing both human renin and human AGT systemically and exhibiting elevated circulating ANG II and hypertension were previously reported and characterized (6, 18, 27, 31). All mice were maintained by backcross breeding to C57BL/6J mice, and double-transgenic mice were generated by breeding heterozygous single-transgenic mice. Identification of transgenic offspring was by PCR amplification of DNA isolated from tail biopsies as described previously (6, 18). All mice were fed standard mouse chow (Teklad LM-485) and water ad libitum. Care of the mice used in the experiments met or exceeded the standards set forth by the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All procedures were approved by the University Animal Care and Use Committee at the University of Iowa.

Endocrinology and human AGT mRNA. Human AGT mRNA and kidney androgen-regulated protein (KAP) mRNA were detected in tissues using methods and probes described previously (6). Plasma levels of ANG II, human AGT, and mouse AGT and urinary levels of human AGT were determined using assays previously reported and validated by us (6, 18, 31). Plasma human AGT was also detected by Western blot analysis using a polyclonal antibody generously provided by Dr. Duane Tewksbury (Marshfield Medical Research Foundation, Marshfield, WI). Five microliters of total plasma were run on a 10% SDS-acrylamide gel, transferred electrophoretically to nitrocellulose, and probed with the polyclonal antibody (1:10,000 dilution). Protein bands were detected using a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody using the Bio-Rad (Hercules, CA) Opti-4CN substrate kit following the directions provided by the manufacturer.

BP measurements. All BP measurements were made in mice in the conscious, freely moving state via an indwelling carotid arterial catheter as previously described (5). Some mice were also instrumented with a jugular vein catheter for intravenous infusion of human renin and losartan. All mice were allowed a minimum of 48 h to recover from surgery before BP experiments were initiated. BP was measured on a Grass model 7 polygraph using Cobe transducers. BP measurements in response to human renin infusion were performed using either partially purified human renin purchased from Scripps Laboratories (San Diego, CA) or purified recombinant human renin provided as a generous gift from Dr. Walter Fischli (Hoffman-LaRoche Pharmaceuticals, Basel, Switzerland) as previously described (31). BP measurements in response to losartan were similarly performed using the doses indicated in the text. Losartan was the generous gift of A. Kim Johnson (University of Iowa).

For experiments involving testosterone administration, mice were first surgically implanted with a carotid catheter and allowed 48 h to recover, and baseline BP (before testosterone) was recorded. Mice were then anesthetized with Metofane, and a testosterone pellet (5 mg designed for 21-day release) was implanted subcutaneously as previously reported (6). BP was again measured 1–4 days after implantation of the pellet. Induction of the transgene was confirmed by Northern blot analysis as previously described (6). In a separate group of mice, testosterone was implanted on *day 0*, and mice were killed on each of *days 1–4* to obtain kidney tissues for analysis of human AGT and KAP mRNA.

Statistical analysis. Data are expressed as means \pm SE. Data were analyzed by ANOVA followed by Student's modified *t*-test with Bonferroni correction for multiple comparisons between means using Systat (version 7.0). SE was determined by the formula $(EMS/n)^{1/2}$, where EMS is the error mean square term from the ANOVA and *n* is the number of mice per group.

RESULTS

To determine if intrarenal production of human AGT contributes to intrarenal ANG II synthesis and is a regulator of systemic BP, we interbred transgenic mice expressing kidney-specific human AGT with transgenic mice expressing human renin to generate a kidney-specific double-transgenic model. We previously demonstrated that our human renin transgenic mice exhibit juxtaglomerular cell-specific expression of human renin mRNA and have circulating active human renin protein at a level commensurate with that found in humans (27). As a positive control for these experiments, we also generated a systemic double-transgenic model by interbreeding the same human renin transgenic mice with mice expressing human AGT systemically (18). Interbreeding of the strains is necessary to reconstitute an enzymatically functional RAS because there is a strict species specificity in the enzymatic reaction between renin and AGT (11, 31). Interestingly, however, no other human RAS component is necessary because the ANG I and ANG II peptides are identical in mice and humans.

Expression of human AGT in the kidney-specific double-transgenic model was restricted to the kidney, whereas its expression in the systemic double-transgenic model was evident in renal and a number of extrarenal tissues, including the liver, the primary site of blood-borne AGT (Fig. 1). BP was measured to determine if kidney-specific expression of human AGT could cause hypertension. BP was measured in conscious unrestrained male and female mice via a carotid

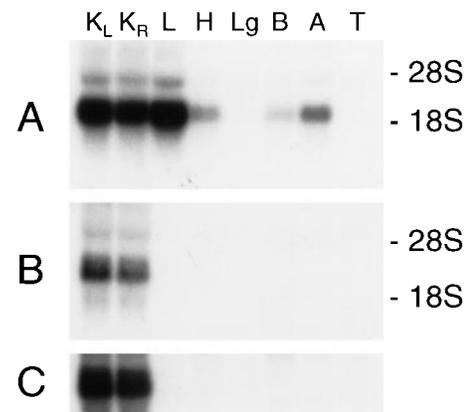


Fig. 1. Kidney-specific expression of human angiotensinogen (AGT) in double-transgenic mice. Northern blot analysis of human AGT expression in male systemic (A) and kidney-specific (B and C) mice is shown for the following tissues: K_L, left kidney; K_R, right kidney; L, liver; H, heart; Lg, lung; B, brain; A, white adipose tissue; T, testes. Expression of kidney-specific double-transgenic mice is from low-expressing line. Positions of 28S and 18S rRNAs are indicated at right. Exposure times are 24 h (A and B) and 80 h (C).

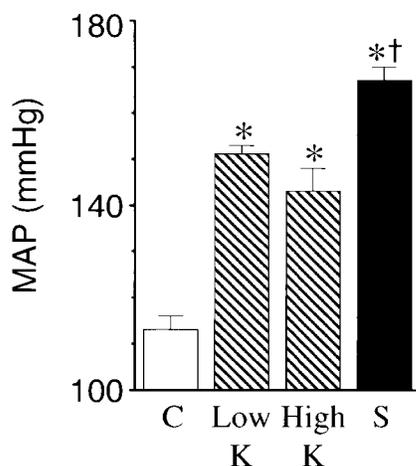


Fig. 2. Basal mean arterial pressure (MAP) in male double-transgenic mice. Basal MAP was recorded in male control mice (C, $n = 8$), kidney-specific double-transgenic mice of low-expressing line (low K, $n = 9$) or high-expressing line (high K, $n = 4$), and systemic double-transgenic mice (S, $n = 13$). * $P < 0.01$ vs. control; † $P < 0.05$ vs. kidney-specific double-transgenic mice.

arterial catheter. As expected, there was no significant difference in the BP of nontransgenic or any single-transgenic mouse. Therefore, nontransgenic and single-transgenic littermates derived from the double-transgenic breedings were combined and used as controls for these studies. BP was significantly elevated in two independent lines of kidney-specific double-transgenic male mice (151 ± 2 and 143 ± 5 mmHg, $P < 0.01$) compared with control nontransgenic male mice (113 ± 3 mmHg, Fig. 2). This increase was intermediate to the BP of systemic double-transgenic mice (167 ± 3 mmHg). The chronic hypertension in the males is consistent with constitutive expression of the KAP promoter-driven human AGT transgene in the kidney. However, under basal conditions there was no detectable human AGT mRNA in kidney of female kidney-specific double-transgenic mice (Fig. 3A). Similarly, the baseline BP of female kidney-specific double-transgenic mice (109 ± 5 mmHg) was the same as nontransgenic mice (113 ± 4 mmHg). A progressive increase in renal human AGT mRNA was evident in female mice after administration of a testosterone pellet (Fig. 3A). The increase in human AGT mRNA was also mirrored by an increase in endogenous KAP mRNA. Testosterone caused an in-

crease in BP in kidney-specific double-transgenic mice similar to the increase in human AGT mRNA (Fig. 3B). BP rose rapidly once the transgene was activated, 19 mmHg after 1 day and 31 mmHg after 2 days, peaking at 40 mmHg (149 ± 4 mmHg) after 3 days. Testosterone did not cause any change in BP in the control group. These results suggest that kidney-specific expression of AGT is a regulator of BP and that kidney-specific overexpression of AGT can cause hypertension.

Previous studies indicate that systemic overexpression of the RAS can cause ANG II-dependent hypertension (9, 18), and it is well established that intravenous infusion of ANG II raises systemic BP. Therefore, we next determined if the hypertension observed in the kidney-specific double-transgenic mice was due to the activation of a selective intrarenal mechanism, as hypothesized, or due to the release (or "leakage") of either intrarenally produced human AGT or ANG II into the systemic circulation. Indeed, kidney-specific synthesis and retention of both human AGT protein and ANG II in this model is a critical factor necessary to test the intrarenal RAS hypothesis. To address this issue we performed a series of important control experiments to rigorously test the kidney specificity of our model. This was accomplished by measuring plasma and urinary human AGT, plasma ANG II, and the BP response to ANG II receptor blockade. Plasma and urinary human AGT were measured in single-transgenic mice lacking human renin, whereas the ANG II measurements were performed in double-transgenic mice. Measurements of circulating endogenous mouse AGT (in pmol/ml) revealed that it was unchanged among any of the strains tested (29.2 ± 7.2 in control, 41.2 ± 7.0 and 24.6 ± 3.7 in 2 lines of kidney-specific males, 21.9 ± 4.8 in 1 line of kidney-specific testosterone-treated females, and 43.2 ± 7.1 in systemic human AGT transgenic mice, $n = 4$, $P > 0.05$). As an important indication of the kidney specificity of the model, there was no significant difference in plasma human AGT between kidney-specific transgenic mice and control mice (Fig. 4A, $P > 0.05$). Elevated levels of human AGT were only detected in the systemic transgenic mice used as positive controls ($P < 0.001$).

Because the level of human AGT detected in the control and kidney-specific mice was at the background of the biochemical assay, we further validated these

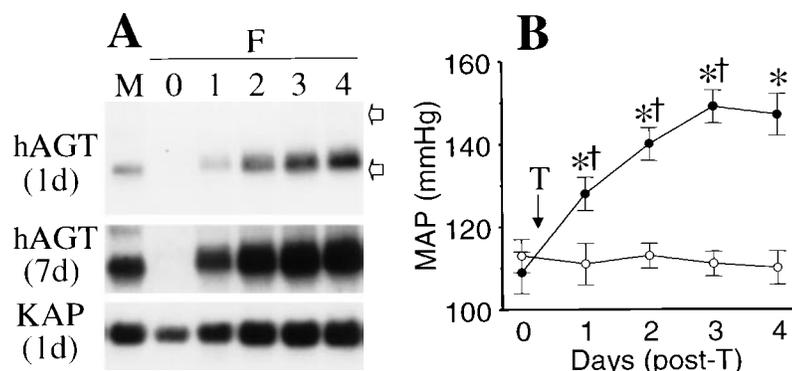


Fig. 3. Androgen regulation of human AGT (hAGT) mRNA and MAP in female double-transgenic mice. A: Northern blot analysis of human AGT mRNA (top and middle) and endogenous kidney androgen-regulated protein (KAP) mRNA (bottom) in kidney from a male double-transgenic mouse (M) and from a female double-transgenic mouse 0–4 days after testosterone administration (F). Top arrowhead, position of 28S rRNA; bottom arrowhead, position of 18S rRNA. Exposure times (in days) are shown for each blot. B: basal MAP was recorded in female control (○, $n = 7$) and kidney-specific double-transgenic mice (●, $n = 6$). MAP was recorded before testosterone (day 0) and on each day for 4 days thereafter. Time of testosterone administration is indicated by arrow. post-T, After testosterone. * $P < 0.01$ vs. control; † $P < 0.05$ vs. MAP recorded the previous day.

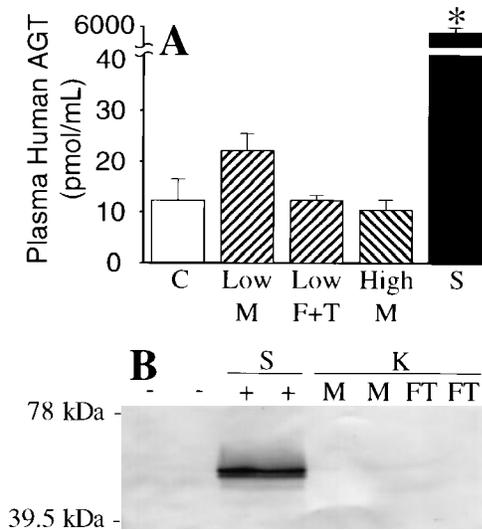


Fig. 4. Circulating human AGT in transgenic mice. *A*: plasma levels of human AGT were measured in control nontransgenic mice (C), in male mice from low-expressing kidney-specific line (low M), in testosterone-induced female mice from low-expressing kidney-specific line (low F + T), in male mice from high-expressing kidney-specific line (high M), and in systemic transgenic mice (S); $n = 4$ in all groups. * $P < 0.001$ vs control. *B*: Western blot analysis of human AGT in total plasma (5 μ l) from 2 control mice (-), 2 systemic mice (S), and 4 low-expressing, kidney-specific (K) mice [2 males (M) and 2 testosterone-induced females (FT)]. Mice from each group are run in adjacent lanes. Molecular mass markers for bovine serum albumin (78 kDa) and carbonic anhydrase (39.5 kDa) are shown at left.

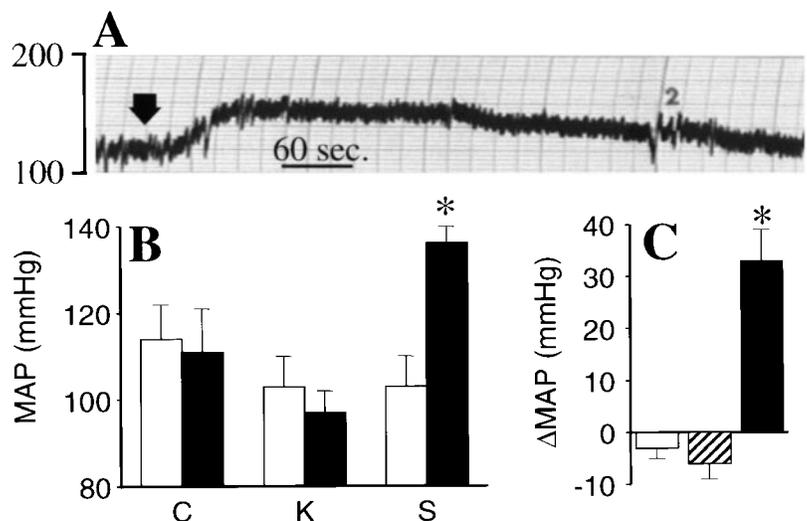
results by assaying for human AGT using both Western blotting and a sensitive bioassay. A clear human AGT protein doublet of the correct molecular mass (likely due to differential glycosylation) was observed in plasma from the systemic human AGT transgenic but not from control or kidney-specific transgenic mice (Fig. 4*B*) (10). To perform the bioassay, transgenic mice were chronically instrumented with carotid arterial and jugular vein catheters for measurement of BP and infusion of human renin, respectively. Because of the species specificity in the processing of AGT by renin (mouse renin cannot cleave human AGT), infusion of purified human renin into mice induces a transient

pressor response only if human AGT is present in the circulation (11). A typical human renin-induced pressor response is shown in Fig. 5*A*. Infusion of purified human renin in either nontransgenic or kidney-specific transgenic mice did not cause a rise in BP because of the absence of circulating human AGT and inability of human renin to cleave endogenous mouse AGT (Fig. 5, *B* and *C*). In contrast, human renin caused a marked pressor response when infused into control transgenic mice containing human AGT systemically. This response was blocked by pretreatment with an angiotensin converting-enzyme inhibitor, indicating it was ANG II dependent (data not shown). Each group still demonstrated an equal capacity to elicit a pressor response to direct infusion of ANG II. These results further demonstrate that kidney-specific expression of human AGT does not lead to the release of human AGT into the systemic circulation.

To confirm whether human AGT protein was indeed being generated intrarenally, we assayed urine from kidney-specific transgenic mice for the presence of human AGT protein. Urine samples were assayed because previous studies in rats indicated the presence of high concentrations of ANG II in proximal tubule fluid, suggesting that AGT released from proximal tubule cells may get rapidly converted to angiotensin peptides (22). Urine samples were collected from both males and testosterone-treated females from two independent lines of kidney-specific single-transgenic mice. Variable but easily detectable human AGT was evident in urine samples from all kidney-specific transgenic mice but not from control nontransgenic littermates, clearly demonstrating that proximal tubule-specific expression of human AGT mRNA leads to human AGT production and release (Fig. 6).

The kidney specificity of ANG II production in this model was first determined by measuring the level of circulating ANG II and then by examining the BP response to acute AT-1 receptor blockade. On the basis of the absence of circulating human AGT, the expression of human AGT mRNA in proximal tubule, and the detection of human AGT protein in urine, we antici-

Fig. 5. MAP response to human renin infusion. *A*: typical pulse pressure tracing of a systemic human AGT transgenic mouse in response to infusion of purified human renin (arrow). *B*: MAP was measured in control mice (C, $n = 5$), kidney-specific single-transgenic mice (K, low-expressing line, $n = 6$), and systemic single-transgenic mice (S, $n = 5$) before (open bars) and after (filled bars) infusion of purified human renin. *C*: peak change in MAP (Δ MAP) induced by human renin infusion. Open bar, control mice; hatched bar, kidney-specific single-transgenic mice; filled bar, systemic single-transgenic mice. * $P < 0.01$ vs. control mice.



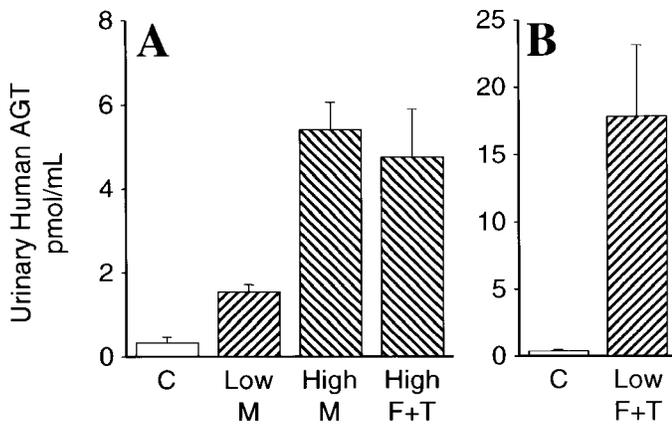


Fig. 6. Urinary human AGT in transgenic mice. Urinary levels of human AGT were measured in control mice (C, $n = 5$) and in kidney-specific single-transgenic mice of the following genders and treatments: male mice of low-expressing line (low M, $n = 3$) or high-expressing line (high M, $n = 3$) and testosterone-treated female mice of high-expressing line (high F + T, $n = 3$) or low-expressing line (low F + T, $n = 4$). A and B are identical except for scale.

pated that any ANG II produced within the kidney would not be released into the circulation. Indeed, there was no significant increase in the level of circulating ANG II in male or testosterone-induced female kidney-specific double-transgenic mice from two independent lines compared with nontransgenic control mice (Fig. 7, $P > 0.05$). As expected, however, circulating ANG II in the control systemic double-transgenic mice was elevated by 3.7-fold ($P < 0.0001$) (18).

Finally, to further distinguish between the BP-elevating effects of intrarenally and systemically derived ANG II, we examined the BP response to an acute intravenous infusion of the selective ANG II type-1 (AT-1) receptor antagonist losartan. Losartan causes a dose-dependent decrease in BP in animal models of ANG II-induced hypertension (16) but not in rodents that have normal levels of circulating ANG II and basal arterial tone. Losartan caused a significant dose-

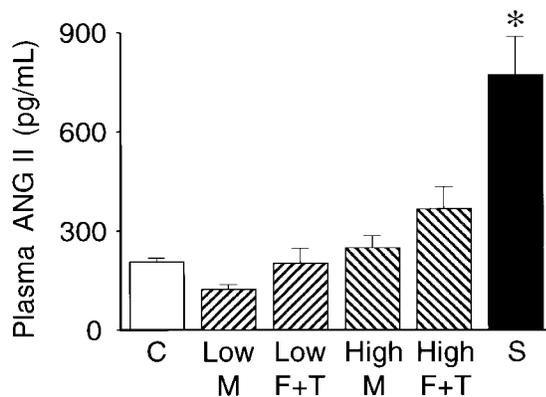


Fig. 7. Circulating ANG II in double-transgenic mice. Plasma ANG II was measured in control (C, $n = 9$), systemic double-transgenic (S, $n = 7$), and kidney-specific double-transgenic mice of the following genders and treatments: male mice of low-expressing line (low M, $n = 8$), testosterone-treated female mice of low-expressing line (low F + T, $n = 4$), male mice of high-expressing line (high M, $n = 5$), and testosterone-treated female mice of high-expressing line (high F + T, $n = 5$). * $P < 0.0001$ vs. control mice.

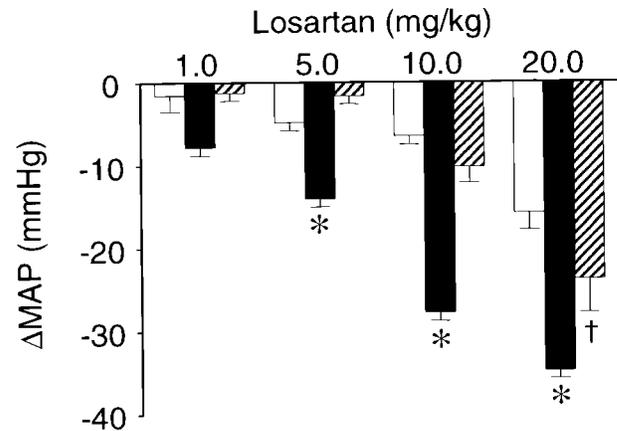


Fig. 8. MAP response to losartan. Peak decrease in MAP in response to indicated doses of losartan is shown. Open bars, control mice ($n = 4$); filled bars, systemic double-transgenic mice ($n = 5$); hatched bars, kidney-specific double-transgenic mice (low-expressing line, $n = 5$). * $P < 0.01$ vs. control and kidney-specific double-transgenic mice; † $P < 0.05$ vs. control mice only.

dependent depressor response in systemic double-transgenic mice, which have elevated plasma ANG II, but had minimal effects on BP in nontransgenic and kidney-specific double-transgenic mice (Fig. 8). Only at the highest dose (20 mg/kg) was the fall in BP significantly greater in kidney-specific double transgenic mice than in control mice. However, the depressor response was still less than that observed in the systemic double-transgenic mice. In addition, the time required for losartan to cause the maximal depressor response (20 mg/kg) was significantly shorter in systemic (5.6 ± 0.3 min, $P < 0.05$) than in kidney-specific (10.0 ± 1.0 min) double-transgenic or control mice (9.5 ± 1.0 min).

All these biochemical, physiological, and pharmacological experiments clearly demonstrate the kidney specificity of our model and strongly suggest that the BP elevation observed is due to the activation of an intrarenal mechanism involving local production of ANG II via intrarenal AGT.

DISCUSSION

The species specificity of the RAS cascade affords a novel opportunity to test important hypotheses regarding tissue RAS by providing a tool to direct the expression of ANG II to a specific site. Targeting production of human AGT to a specific cell type will result in local synthesis of ANG II only if human renin is also present. In this study we demonstrate that expression of human AGT specifically in the kidney leads to systemic hypertension even in the absence of circulating human AGT protein and with normal plasma ANG II levels. We employed the KAP promoter to restrict human AGT to the proximal convoluted tubule of the kidney. This promoter shares the same cell specificity and androgen responsiveness as the endogenous AGT promoter in kidney but is not transcriptionally active in extrarenal tissues (6, 20). Direct enzymatic assay, Western blot analysis, and lack of a BP response to human renin infusion clearly demonstrated that human AGT pro-

duced in the proximal tubule does not get released into the systemic circulation. Moreover, the absence of elevated plasma ANG II and the lack of a depressor response to doses of losartan that effectively reduce BP in the systemic model demonstrate that ANG II produced from proximal tubule-derived human AGT is not released into the systemic circulation.

Although both the kidney-specific and systemic double-transgenic models exhibit hypertension, differences in their BP sensitivity to acute losartan are indicative of distinct mechanisms of hypertension. In the systemic model, circulating ANG II levels are high and acute ANG II receptor blockade impairs the vasoconstrictor responses to ANG II. It is also possible that acute administration of losartan in the systemic model may block some effects mediated by ANG II on the central nervous system. Indeed, we have recently demonstrated an important role for the central nervous system in the mechanism producing hypertension in the systemic model (5). It is interesting to note that in the kidney-specific model both the timing of the response and the shift in the BP response to a higher dose of losartan are consistent with the filtering actions of the kidney, where only a fraction of the blood is filtered during a single pass through the nephron. Therefore, it is possible that an effective intratubular concentration of losartan may have only been achieved at the highest dose. ANG II regulates renal blood flow, glomerular filtration rate, and tubuloglomerular feedback and mediates a number of direct and indirect effects on sodium homeostasis in the kidney. ANG II has direct effects on sodium transport in the early nephron by stimulating sodium-hydrogen exchange in proximal tubule and has indirect effects in the late nephron by regulating synthesis of epithelial sodium channels by aldosterone (3, 24). This supports the hypothesis that hypertension in these mice may be due to alterations in sodium or fluid homeostasis, perhaps through alterations in these transport mechanisms. Such effects appear to be a common underlying mechanism causing high BP in a number of human genetic hypertensive syndromes (26, 28). Moreover, these results would be consistent with our observation that in females testosterone caused an induction of transgene mRNA that reached male levels within 1 day, whereas the maximum increase in BP required 3 days.

Our data further suggest that the proximal tubule is the site of ANG II production and action in this model. This is supported by the specific expression of the human AGT transgene in the proximal tubule (6, 13), the localization of ANG II in fluid from the proximal tubule (22), the absence of circulating human AGT measured both biochemically and physiologically, and the presence of human AGT protein in urine samples from kidney-specific transgenic mice. These data suggest that AGT produced by proximal tubule cells is subject to polarized transport through the apical membrane and secretion into the lumen, the location of numerous ANG II AT-1 receptors (7). Additional studies will need to be performed to formally address whether release of AGT occurs through the basolateral mem-

brane into the kidney interstitium, although the absence of plasma human AGT and ANG II tends to argue against basolateral secretion.

Our data support the conclusion that systemic hypertension observed in the kidney-specific model is due to activation of a selective intrarenal BP-elevating mechanism involving local production of both AGT and ANG II. The importance of a tissue-restricted intrarenal RAS in BP regulation is gaining support by studies of essential hypertension in humans. Inhibitors of ANG II synthesis or action can be effective antihypertensive agents in patients with normal or low plasma renin activity, a clinical measure of the endocrine RAS, suggesting that inhibition of tissue RAS may lower BP (2, 8). Recent studies have implicated AGT in the genetic basis of hypertension and preeclampsia (15, 30). One haplotype of AGT (−6A/235T), found associated with hypertension, includes an allelic variant in the promoter that increases its activity in transfected cells (14). Moreover, higher expression of −6A/235T mRNA was reported in women heterozygous for this haplotype (21), suggesting that hypertensive patients carrying this haplotype may also exhibit higher expression of AGT in kidney. Indeed, a group of hypertensive patients known as nonmodulators are thought to exhibit increased intrarenal ANG II (23), and subjects carrying the −6A/235T haplotype share phenotypic similarities with nonmodulators (12). Consequently, the pathophysiological effects of AGT and its variants may act tissue specifically, and therefore genetic abnormalities affecting the expression of tissue-restricted RAS need to be explored as important mechanisms of essential hypertension.

In conclusion, these results support the hypothesis that a tissue-specific RAS cascade exists in the kidney of this transgenic model and that this system may play an important role in BP and renal homeostasis in this model. Moreover, the models described herein should provide new tools to further examine the intrarenal RAS. The fortuitous androgen responsiveness of the transgene should provide an opportunity to perform both acute and chronic experiments with an aspect of temporal control that does not currently exist in other transgenic or knockout models of hypertension. Finally, the concept that there may be functional differences between an endocrine and tissue-restricted biological system is not unique to the RAS. Often, however, differentiating between these systems can be technically challenging because of the specificity of pharmacological inhibitors or difficulties in their delivery to appropriate cell types. The cell-specific targeting described herein coupled with new advances in genetic methodology allowing us to regulate transgene expression or generate cell-specific knockouts will likely provide important new experimental paradigms to distinguish between circulating and tissue-based physiological systems.

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