Renal proximal tubule angiotensin AT1A receptors regulate blood pressure

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Submitted 10 March 2011; accepted in final form 9 July 2011

THE RENIN-ANGIOTENSIN SYSTEM (RAS) is well recognized for its contributions to blood pressure regulation and sodium homeostasis, and all components of the RAS are expressed within the kidney. Within the proximal convoluted tubule (PCT), angiotensinogen (AGT) and angiotensin converting enzyme (ACE) have been identified on the brush border of tubular epithelial cells (21). ANG II type 1A receptors (AT1AR) are localized in both apical and basolateral membranes of the PCT, with a higher density compared with the other nephron segments (20). Previous studies have also demonstrated that the ANG II concentration in the PCT is much higher than in plasma (10). These data suggest that local production and action of ANG II in the PCT may play a significant role in sodium retention and blood pressure.

The effects of ANG II on sodium transport in the PCT have been studied intensively. In the isolated PCT, ANG II stimulates sodium transport at physiological concentrations, primarily by activation of the sodium hydrogen exchanger 3 (NHE3) (19, 39). In vivo studies using transgenic and knockout mouse models of RAS components have also implicated PCT ANG II in blood pressure regulation. We previously generated two double-transgenic mouse models expressing human AGT (hAGT) under the control of the kidney androgen-regulated protein (KAP) promoter, which directs PCT cell-specific gene expression in an androgen-responsive manner (14, 15). In the first model, human renin expression was restricted to renal juxtaglomerular cells, its classic site of synthesis in the kidney. Juxtaglomerular-specific human renin combined with proximal tubule-specific expression of hAGT resulted in significantly elevated blood pressure, even though plasma ANG II concentrations were unchanged (13, 22). In the second model, human renin and hAGT were coexpressed in proximal tubule cells (each under the control of the KAP promoter) resulting in elevated arterial pressure (23). These data provided strong evidence that ANG II generated in the PCT and acting in the kidney could raise blood pressure. However, because the receptors for ANG II are expressed along the entire nephron (20), it is possible that the sodium-retention and blood pressure effects evident in these animals were mediated through ANG II action at both PCT and downstream tubular segments. Recent studies by Gurley et al. (17) provide compelling data implicating an important role for PCT AT1AR in the regulation of arterial pressure.

In this study, we examined ANG II action specifically within the PCT. This was accomplished through the generation and characterization of two new transgenic mouse models. First, we developed mice expressing a ligand-independent, chronically active AT1AR mutant under transcriptional control of the PCT-specific, androgen-dependent, second generation KAP promoter (KAP2-AT1AR-N111G mice). Second, similar to the recent study by Gurley et al. (17), we developed mice with PCT-specific deletion of AT1AR (KAP2-AT1AR-KO mice), herein employing the androgen-dependent KAP2-iCre transgenic model previously reported by us (27). Subsequent characterization of these mice supports the hypothesis that AT1AR on the PCT regulate blood pressure in vivo.

MATERIALS AND METHODS

Generation of KAP2-AT1AR-N111G and KAP2-AT1AR-KO mice. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Iowa. To generate the KAP2-AT1AR-N111G transgenic mice, a cDNA fragment encoding rat AT1AR (N111G) was inserted into the Not I site of the KAP2 construct. The KAP2 construct is composed of 1,542 bp of KAP promoter fused to a gutted coding region of the hAGT gene and has been shown to drive PCT-specific gene expression in an androgen-inducible manner (5). The rat AT1AR (N111G) cDNA fragment was amplified from the vector pRC-CMV-ATIR (provided by Dr. Walter...
Proximal tubule-specific AT1AR knockout mice (KAP2-AT1AR-KO) were generated by crossing AT1ARfloxed mice on an inbred C57BL/6J genetic background obtained from the University of Kentucky (35) with KAP2-icre transgenic mice, which were originally generated by our laboratory (27). Both models are now available from the Jackson Laboratory. All mice were studied at 4–6 mo of age. We examined both male and female mice in this study (except for Western blot analysis and radioligand binding, which were performed exclusively in female mice). However, a rise in arterial pressure in KAP2-AT1AR-KO-examined both male and female mice in this study (except for Western blot analysis and radioligand binding, which were performed exclusively in female mice). Therefore, for consistency, the majority of the data presented here are from females treated with testosterone. We clearly denote those experiments where males were included.

DNA and RNA isolation and PCR. Mice were killed by CO2 asphyxiation followed by cervical dislocation. Tissues were harvested, snap frozen in liquid nitrogen, and stored at −80°C. Tissues were cut into small pieces and digested in DNA lysis buffer (10 mM Tris·Cl pH 7.5, 0.1 mM EDTA) at a concentration of 2 ng/µL, and microinjected into the pronuclei of fertilized oocytes from C57BL/6J X SJL/J F2 mice. Among seven founder mice, line 43058/2, which exhibited the most restricted tissue-specific expression profile, was backcrossed onto a C57BL/6J background for at least four generations before experimental studies. PCR was performed using genomic DNA isolated from tail clip for genotyping. Primer sequences used for genotyping and expression profiling are provided in Supplemental Table S1 (supplemental data are posted with the online version of this article).

Testosterone pellet implantation and ANG II infusion. Mice were anesthetized with isoflurane, and a testosterone pellet (10 mg; Innovative Research of America) was implanted subcutaneously in the mouse back using a trocar (Innovative Research of America). Mice were infused with ANG II (800 ng·kg⁻¹·min⁻¹, 10 days) by using osmotic minipumps (model 1002, Alzet) implanted subcutaneously through a small incision during anesthesia with ketamine (87.5 mg/kg) and xylazine (12.5 mg/kg). All studies were performed on the animals (nontransgenic, AT1AR transgenic, or knockout) after 10 days of testosterone administration, unless otherwise indicated.

Tail cuff plethysmography and radiotelemetry. Systolic blood pressure (SBP) and heart rate were measured using a computerized tail-cuff system (Visitech Systems). Mice were acclimated to the system for at least 5 days prior to measurements for 5 or 10 days. During the recording, mice were placed in restrainers on a heated platform. Care was taken to avoid overheating the mice or excessively restricting their movement. Ten preliminary cycles were performed followed by 30 measurement cycles. Values deviating more than two standard deviations from the mean were considered outliers and were eliminated from the analysis. The average of 5 or 10 days recording was used for each mouse.

Blood pressures were measured in conscious mice by using a radiotelemeter (model TA11PA-C10; Data Science International). Mice were anesthetized with ketamine (87.5 mg/kg) and xylazine (12.5 mg/kg), and the telemeter was inserted as described previously (24). After surgery, mice were kept on heating pads until fully awake. They were housed in individual cages for 10 days of recovery. SBP, diastolic blood pressure, mean arterial blood pressure, and heart rate were recorded using Dataquest ART 3.1 (Data Sciences International). Recordings were taken for 20 s every 5 min for 5 days. During the recordings, the mice were exposed to 12:12-h light-dark cycle and had access to water and food ad libitum. The measurements were averaged into 1-h and 12-h mean values. Recordings with pulse pressures < 20 mmHg or an absence of circadian rhythms were eliminated. The average of 5 days of recording was used for each mouse.

Metabolic studies and chemistry. Mice were placed in metabolic cages (Nalgene) and had free access to chow (Teklad 7013, NIH-31 modified 6% mouse diet) and tap water. After 1 day of acclimation, food intake, fluid intake, and urine excretion were recorded for 2 days. Some mice had access to a two-bottle choice of water or normal saline. Bottle positions were switched daily to account for side bias. The mean of 2 days of measurements was used for comparisons. Urine collected from metabolic cages was centrifuged to remove food debris. Osmolality was measured with the freezing point depression method (FISKE 2400 multi-sample osmometer), and sodium and potassium concentrations were assessed by flame photometry (Instrumentation Laboratory).

Radioligand binding assays. Angiotensin type 1 and 2 receptor density was measured using quantitative in vitro autoradiography using 125I-[Sar1, Ile8] ANG II as radioligand as described (25, 38). Briefly, 20-µm sections were cut through the midportion of the kidney by using a cryostat and mounted onto gelatin-coated glass slides. Consecutive sections from each kidney were incubated for 1 h at room temperature in phosphate-buffered NaCl containing 125I-[Sar1, Ile8] ANG II (~90 PM) alone (total binding) or in the presence of 1µM ANG II (nonspecific binding), 1 µM losartan (to displace binding from AT1 receptors, thus revealing AT2 receptors) or 10 µM PD123319 (to reveal AT1 receptors). The sections were then washed, dried, and exposed, along with radioactivity standards, to X-ray film (UM-MA HC medical X-ray film; Fuji). Binding densities were analyzed using a computerized imaging system and optical densities were converted (dpm/mm²; MCID Imaging). Ten samples of standard size were measured from the glomeruli and cortex in each section (n = 3–4 animals per genotype). Nonspecific binding was averaged and subtracted to give specific binding for total, AT1 and AT2 receptors. The measurements from each animal were then averaged according to genotype and expressed as means ± SE.

Statistical analysis. Data are presented as means ± SE. For comparisons of metabolic parameters and gene expression levels between
groups, Student’s t-test was used. For comparisons of blood pressure and urine osmolalities, two-way repeated-measures ANOVA was used, followed by a Fisher least significant differences multiple-comparison correction. For real-time PCR, fold changes were calculated using the Livak method. \( P \) values < 0.05 were considered to represent statistical significance.

RESULTS

To investigate the function of the AT1ARs in the renal PCT, we overexpressed a constitutively active form (N111G) of the rat AT1AR that is active in the absence of ANG II. This form of AT1AR, when injected bilaterally into the rostral ventrolateral medulla of the brain, resulted in increased blood pressure (1). PCT specificity of the transgene was achieved using a chimeric construct containing the KAP promoter and a gutted nonfunctional portion of the hAGT gene. We have reported that this chimeric construct directs strong androgen-inducible and PCT-specific expression (Fig. 1A) (14).

Because of androgen regulation of the transgene, we first examined expression of the transgene in kidneys of female mice in the presence and absence of testosterone. As expected, expression of the transgene in the kidney was induced strongly (~9-fold) by exogenous testosterone (data not shown). Compared with nontransgenic mice, which by definition do not express the transgene, there was nearly a 100-fold increase in detection of transgene mRNA in kidney from testosterone-treated females (Fig. 1B). In these transgenic mice, there was a 2.8-fold increase in total (transgene and endogenous) AT1AR mRNA. Interestingly, increases in total AT1AR mRNA occurred in the face of a 2.6-fold decrease in endogenous mouse AT1AR mRNA (Fig. 1B). Consequently, there was no detectable increase in total ANG II binding in the kidney according to radioligand binding assays (Fig. 2). The radioligand was displaced by losartan, but not by PD123319, confirming that these results reflect ANG II binding to AT1, not AT2 receptors.

Combined, these data suggest that the majority of endogenous...
wild-type receptors had been replaced with constitutively active receptors driven from the transgene. Consistent with this, under basal conditions, we observed increased signaling downstream of AT1ARs as indicated by an approximate twofold increase in phosphorylated ERK in the renal cortex of five testosterone-treated female transgenic mice compared with five testosterone-treated nontransgenic littermates when normalized either to total ERK or GAPDH (Fig. 1C). Although driven by a PCT-specific promoter, expression of the transgene was also observed in the brain and ovary (Fig. 1D). There was no detectable expression in the lung, heart, liver, or submandibular gland. Similar results were obtained in males (data not shown).

As a complement to the above model, we generated mice with PCT-specific depletion of endogenous AT1AR. This was done by breeding KAP2-Cre transgenic mice (27) for two generations with AT1ARflox/flox mice (35). Using a sensitive PCR-based assay on genomic DNA from kidney, we show that the Cre-recombination product (null or ∆ allele) was detected only in kidney from mice carrying both Cre-recombinase and a floxed allele, and that the level of the null allele was increased in female mice treated with testosterone (Fig. 3A). The null allele was detected in genomic DNA from kidney, but not brain, lung, heart, submandibular gland, liver, or ovary, suggesting a high degree of tissue specificity (Fig. 3B). Overall, decreases in total AT1AR mRNA in response to Cre-recombinase averaged 30% of control mice. Despite decreases in AT1AR expression, there was no remarkable decrease in ANG II receptor binding detected in the cortex or glomeruli suggesting regulation of the AT1AR binding pool (Fig. 4).

We next measured blood pressure in male and female KAP2-AT1A-N111G transgenic mice by tail cuff. There was a 10 mmHg rise in blood pressure in female transgenic mice, but not littermate controls after induction of transgene expression by testosterone (Fig. 5A). In males, although there was a marked increase in expression of the transgene after testosterone administration (data not shown), there was no difference in blood pressure in male transgenic mice, either with or without testosterone, compared with nontransgenic littermates (Fig. 5A). ANG II infusion

**Fig. 2. ANG II receptor expression in kidney using radioligand binding assay.** ANG II binding in whole kidney from KAP2-AT1AR-N111G Tg female mice (KAP2-AT1A) administered testosterone and their NT controls. Total, binding in absence of receptor antagonists; NIS, nonspecific binding; losartan, binding after specific blockade of ANG II AT1 receptors; PD, binding after specific blockade of ANG II AT2 receptors with PD123319.

**Fig. 3. Characterization of proximal tubule-specific AT1AR-depleted mice.** A: PCR for AT1AR genomic DNA isolated from kidney of KAP2-Cre X AT1ARflox/flox (F/F) mice, and heterozygous (F/+ and wild-type (+/+)) littermates treated (+) or untreated (−) with testosterone. All mice were positive for KAP2-Cre transgene. The position of the PCR products from wild-type AT1A (WT) and recombined genomic DNA (Δ) are indicated. B: PCR for genomic DNA isolated from brain (B), heart (H), lung (Lu), spleen (S), liver (Lv), kidney (K), and ovary (O) of a KAP2-Cre X AT1ARflox/flox mouse treated with testosterone.
caused a similar increase in blood pressure in both transgenic and nontransgenic controls and differences between genotypes were retained (Fig. 5B).

We next measured arterial pressure using radiotelemetry in testosterone-treated female KAP2-AT1AR-N111G transgenic mice, KAP2-cre X AT1Aflox/flox, and their littermate controls (Fig. 6). First, we determined that there were no differences in arterial pressure or heart rate comparing control mice from the transgenic study with the control mice from the knockout study. Thus, those datasets were combined. There was a modest increase in systolic and mean arterial pressure in transgenic mice compared with controls that occurred at the peak of the blood pressure response during the dark cycle. This increase was accompanied by a transient increase in heart rate. Similarly, there were small transient decreases in mean arterial pressure in PCT-specific KAP2-Cre X AT1Aflox/flox mice compared with controls. Differences in arterial pressure were consistently observed when we compared KAP2-AT1AR-N111G transgenic mice with PCT-specific knockout mice suggesting a dose-dependent response to AT1AR expression. In aggregate, the differences in arterial pressure between transgenics and controls (15.4 mmHg) and knockouts and controls (13.4 mmHg) were slightly greater during the dark cycle than the light cycle (10.0 mmHg in transgenics and 12.2 mmHg in knockouts). Similarly, there were significant differences between transgenic and PCT-specific knockout mice during the light (21.9 mmHg systolic) and dark (28.8 mmHg systolic) phases for systolic and mean arterial pressure and during the dark phase for diastolic pressure. Similar results were obtained when a small number of testosterone-treated male control (n = 3) and knockout mice (n = 2) were added to this data set (Supplemental Fig. S1).

To assess metabolic and renal phenotypes, testosterone-treated female mice, including transgenic and nontransgenic littermate controls, were placed individually in metabolic cages and given a two-bottle choice (0.9% saline or water). There were no significant differences in body mass, food or water intake, sodium preference, total sodium intake, urinary sodium or potassium excretion, urinary volume, or urinary osmolality between groups (Table 1). Similarly, for KAP2-Cre X AT1Aflox/flox female mice treated with testosterone, no significant differences in metabolic or renal parameters were observed when mice were placed in metabolic cages with a single-choice protocol (water only) (Table 2). To address the issue of different protocols (i.e., one- vs. two-bottle), we also examined KAP2-Cre X AT1Aflox/flox mice given a two-bottle choice and observed no significant differences compared with the control group (data not shown).
Quantitative PCR was performed to examine whether constitutive activation or deletion of AT1AR in the PCT altered expression of the other RAS components in the kidney of transgenic mice. There were no changes in renin, AGT, ACE, ACE2, or AT1BR expression. Interestingly, a marked decrease in AT2 receptor expression was noted. In the knockout mice, a decrease in endogenous AT1AR and ACE mRNA expression was observed. A trend toward decreased AT2R mRNA was noted but did not reach statistical significance.

Given the importance of sodium transporters in blood pressure regulation, we measured mRNA expression of the major sodium transporters in kidney from testosterone-treated female mice to assess whether constitutive activation or loss of PCT AT1A receptors altered their expression (Table 4). There was no change in the expression of the NHE3, the three splicing forms of the sodium potassium transporter type 2 (NKCC2A, NKCC2B, NKCC2F), the sodium chloride cotransporter NKCC2F), the sodium chloride cotransporter forms of the sodium potassium transporter type 2 (NKCC2A, NKCC2B, NKCC2F), the sodium chloride cotransporter forms of the sodium potassium transporter type 2 (NKCC2A, NKCC2B, NKCC2F), the sodium chloride cotransporter forms of the sodium potassium transporter type 2 (NKCC2A, NKCC2B, NKCC2F).
Table 3. Kidney RAS mRNA

<table>
<thead>
<tr>
<th>Gene</th>
<th>ΔCT</th>
<th>Fold of WT</th>
<th>ΔCT</th>
<th>Fold of WT</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NT, n = 4</td>
<td>Transgenic, n = 4</td>
<td>NT, n = 5</td>
<td>Knockout, n = 4</td>
<td></td>
</tr>
<tr>
<td>Renin</td>
<td>3.39 ± 0.39</td>
<td>1.00 (0.82–1.21)</td>
<td>3.66 ± 0.16</td>
<td>0.83 (0.73–0.93)</td>
<td>0.553</td>
</tr>
<tr>
<td>AGT</td>
<td>3.85 ± 0.37</td>
<td>1.00 (0.84–1.18)</td>
<td>3.90 ± 0.24</td>
<td>0.97 (0.84–1.12)</td>
<td>0.912</td>
</tr>
<tr>
<td>ACE</td>
<td>1.48 ± 0.36</td>
<td>1.00 (0.85–1.18)</td>
<td>2.10 ± 0.19</td>
<td>0.65 (0.58–0.73)</td>
<td>0.177</td>
</tr>
<tr>
<td>ACE2</td>
<td>5.83 ± 0.08</td>
<td>1.00 (0.97–1.04)</td>
<td>5.30 ± 0.56</td>
<td>1.44 (1.07–1.94)</td>
<td>0.319</td>
</tr>
<tr>
<td>AT1A</td>
<td>8.69 ± 0.54</td>
<td>1.00 (0.78–1.28)</td>
<td>10.78 ± 0.18</td>
<td>0.24 (0.21–0.26)</td>
<td>0.010</td>
</tr>
<tr>
<td>AT1B</td>
<td>15.85 ± 0.50</td>
<td>1.00 (0.80–1.26)</td>
<td>16.68 ± 0.80</td>
<td>0.56 (0.34–0.93)</td>
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<tr>
<td>AT2</td>
<td>13.26 ± 0.58</td>
<td>1.00 (0.77–1.31)</td>
<td>15.16 ± 0.39</td>
<td>0.27 (0.21–0.34)</td>
<td>0.035</td>
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</table>

Values are means ± SE. *Student’s t-test. Numbers in parentheses represent ±1 SE. Fold induction were calculated using the Livak method. CT, threshold cycle; WT, wild-type; RAS, renin-angiotensin system.

(NCC), the three subunits of the epithelial sodium channel (ENaC-α, ENaC-β, and ENaC-γ), the sodium potassium ATPase (NaKATPase), or the sodium phosphorous transporter 2 (NaPi2) in the kidney of transgenic mice. On the contrary, a significant decrease in NKCC2A, NKCC2F, and NCC expression was detected in the kidneys of knockout mice.

Finally, since expression of the KAP2-AT1AR-N111G transgene was evident in the brain, we assessed whether there was altered expression of other RAS components in the brain from testosterone-treated female mice (Table 5). There was no change in renin, AGT, ACE, endogenous AT1AR, or AT1BR mRNA. However, there was a significant increase in AT2 receptor mRNA in the brains of transgenic mice. There were no significant changes in expression of any RAS gene in the brain of the PCT AT1AR-depleted mice.

DISCUSSION

Components of the RAS are present in the systemic circulation and are expressed in many different tissues and cell types; however, it has been difficult to separate the contribution of ANG II acting via its receptors in the vasculature from its actions in other cell types such as renal tubular cells. In the present study using two different mouse models, we show that PCT AT1AR play a role in the regulation of baseline blood pressure. PCT-specific overexpression of a constitutively ac-

Table 4. Kidney sodium transporter mRNA

<table>
<thead>
<tr>
<th>Gene</th>
<th>ΔCT</th>
<th>Fold of WT</th>
<th>ΔCT</th>
<th>Fold of WT</th>
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<td>Transgenic, n = 6</td>
<td>NT, n = 5</td>
<td>Knockout, n = 4</td>
<td></td>
</tr>
<tr>
<td>NHE3</td>
<td>3.95 ± 0.77</td>
<td>1.00 (0.56–1.78)</td>
<td>4.93 ± 0.72</td>
<td>0.51 (0.31–0.84)</td>
<td>0.388</td>
</tr>
<tr>
<td>NKCC2-A</td>
<td>4.45 ± 1.04</td>
<td>1.00 (0.46–2.16)</td>
<td>4.92 ± 0.79</td>
<td>0.72 (0.42–1.25)</td>
<td>0.742</td>
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<tr>
<td>NKCC2-B</td>
<td>5.86 ± 0.90</td>
<td>1.00 (0.51–1.95)</td>
<td>6.42 ± 0.63</td>
<td>0.68 (0.44–1.05)</td>
<td>0.642</td>
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<tr>
<td>NKCC2-F</td>
<td>3.23 ± 0.69</td>
<td>1.00 (0.60–1.67)</td>
<td>3.83 ± 0.65</td>
<td>0.66 (0.42–1.03)</td>
<td>0.550</td>
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<tr>
<td>NCC</td>
<td>1.99 ± 1.02</td>
<td>1.00 (0.47–2.13)</td>
<td>2.50 ± 0.93</td>
<td>0.70 (0.37–1.33)</td>
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<tr>
<td>ENaC-α</td>
<td>3.94 ± 0.64</td>
<td>1.00 (0.62–1.60)</td>
<td>4.50 ± 0.75</td>
<td>0.68 (0.40–1.14)</td>
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<td>ENaC-β</td>
<td>4.95 ± 0.70</td>
<td>1.00 (0.60–1.68)</td>
<td>6.01 ± 0.95</td>
<td>0.48 (0.25–0.93)</td>
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<tr>
<td>ENaC-γ</td>
<td>4.53 ± 0.77</td>
<td>1.00 (0.56–1.77)</td>
<td>4.87 ± 0.65</td>
<td>0.79 (0.50–1.24)</td>
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<td>NaK-ATPase</td>
<td>0.22 ± 0.83</td>
<td>1.00 (0.54–1.85)</td>
<td>1.75 ± 0.82</td>
<td>0.35 (0.20–0.61)</td>
<td>0.225</td>
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<td>NaPi2</td>
<td>−0.76 ± 0.71</td>
<td>1.00 (0.59–1.69)</td>
<td>−0.95 ± 0.97</td>
<td>1.14 (0.58–2.24)</td>
<td>0.875</td>
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</table>

Values are means ± SE. *Student’s t-test. Numbers in parentheses represent ±1 SE. Fold induction were calculated using the Livak method.
Renin 15.44 ± 0.86 1.00 (0.55–1.81) 15.10 ± 0.60 1.27 (0.84–1.93) 0.713
AGT 3.05 ± 0.24 1.00 (0.85–1.18) 3.09 ± 0.56 0.97 (0.66–1.43) 0.947
ACE 6.71 ± 0.16 1.00 (0.90–1.12) 5.89 ± 0.44 1.77 (1.31–2.39) 0.116
ACE2 12.15 ± 1.01 1.00 (0.50–2.01) 11.30 ± 0.37 1.80 (1.40–2.32) 0.350
AT1A 15.37 ± 0.22 1.00 (0.89–1.13) 14.95 ± 0.75 1.34 (0.80–2.25) 0.658
AT1B 14.08 ± 0.36 1.00 (0.78–1.28) 13.65 ± 0.53 1.35 (0.98–1.85) 0.504
AT2 12.20 ± 0.13 1.00 (0.89–1.12) 10.39 ± 0.35 3.50 (2.75–4.47) 0.006

Values are means ± SE. *Student’s t-test. Numbers in parentheses represent ± 1 SE. Fold induction were calculated using the Livak method.
The reason for this is not clear, but may be due to a sex-specific difference in the response to increased ANG II signaling. In our experiment, we observed a decrease in renal AT2 receptor mRNA in female, but not in male transgenic mice (data not shown). In male mice, the maintenance of AT2R levels, and intact signaling via those receptors, may act as a buffer against the blood pressure raising actions of the AT1AR.

**PCT-specific AT1AR depletion.** AT1AR knockout and renal transplantation studies have demonstrated that complete loss of AT1A receptors from the kidney reduces blood pressure by about 20 mmHg (11). This is not a uniform finding, as smaller decreases in SBP were reported in a different AT1AR-deficient mouse model (12). Herein we report a 12–13 mmHg decrease in arterial pressure in testosterone-treated female KAP2-iCRE X AT1AR<sup>Flox/Flox</sup> mice exhibiting PCT-specific AT1AR deficiency. The 70% decrease in AT1AR mRNA and the presence of a cre-recombined null allele in genomic DNA from the kidney supports the effectiveness and efficiency of the Cre-recombinase-mediated deletion. Surprisingly, however, there was no detectable decrease in ANG II radioligand binding in these mice, a finding that remains unexplained. This stands in contrast to the recent report by Gurley et al. (17) that tested the same hypothesis using a similar (but not identical) AT1AR<sup>Flox/Flox</sup> model bred with PEPCK-cre transgenic mice. Normally, the PEPCK gene is abundantly expressed in the liver, kidney, and adipose tissue, and thus under most circumstances, utilizing the PEPCK promoter as the driver for cre-recombinase would seem to be counterintuitive as it would target both hepatic and adipose AT1AR. Apparently, they employed a unique transgenic line that expresses cre-recombinase specifically in renal PCT cells (34). Indeed, they showed PCT-specific activity of the PEPCK-cre in the kidney, but did not report whether there was activity in other AT1AR expressing tissues that could influence arterial pressure. It is notable that use of the PEPCK promoter as a driver of Cre-recombinase led to much more effective ablation (40%) of AT1R binding sites in the renal cortex when compared with two control mice than it was in our study employing the androgen-responsive KAP promoter. Interestingly, despite this important difference, both groups likewise measured a 10 mmHg decrease in baseline arterial pressure. Gurley et al. (17) extended their study by reporting that PCT AT1AR regulates fluid reabsorption and PCT AT1AR-deficient mice are protected from ANG II-induced hypertension. Future studies would have to be performed to assess whether PCT AT1AR deficiency using the KAP2-iCre model is similarly effective in protecting from ANG II or other forms of hypertension.

In a further point of similarity between the studies, there was no change in expression of NHE3, the sodium phosphate cotransporter (NaPi2), subunits of the epithelial sodium channel, or the sodium potassium ATPase under baseline conditions. We noted a significant decrease in expression of several subunits of the Na-K-2Cl cotransporter (NKCC2) in our PCT AT1AR-deficient mice. Interestingly, Gurley et al. (17) reported changes in the abundance of NHE3 in response to ANG II infusion, an effect augmented by PCT-specific AT1AR ablation. Thus, whereas PCT AT1AR deficiency may not alter the abundance of major renal sodium transporters under baseline conditions, PCT AT1AR may play important roles as regulators of renal transporters under conditions of AT1AR activation or under other pathological conditions. In our study, it remains unclear whether the modest decreases in ACE and AT2R mRNAs in the kidney of PCT-AT1AR-deficient mice played a mechanistic role in the regulation of arterial pressure.

**Limitations of the study and remaining questions.** One of the most obvious limitations to our study was the apparent expression of the transgene in extra-renal tissues such as the ovary and brain. Although the consequences of ovarian expression of constitutively active AT1AR remains undefined, its expression in the brain could have obvious consequences for the regulation of arterial pressure. Remarkably, the chimeric KAP promoter (termed KAP2) originally developed by us (5) has been used by us and others to target PPARα (28), AGT (37), and renin (23) without notable expression in the brain. AT1 receptors in the brain have long been known to be an important regulator of arterial pressure, hydromineral balance, vasopressin release, and sympathetic drive, and therefore a contribution of brain AT1AR in the KAP2-AT1AR-N111G model cannot be excluded. In the case of the KAP2-N111G-AT1AR transgenic model, expression of the transgene in the brain led to increased expression of AT2 receptors in the brain. AT2 receptors largely counterbalance the effects of the AT1R. For example, intracerebroventricular injection of the AT2R antagonist PD123319 augments the pressor response to central ANG II, and the pressor response to intracerebroventricular ANG II is augmented in AT2R-deficient mice (29). Thus, overexpression of AT2R in the brain may have had the effect of diminishing arterial pressure in the overexpression model. On the contrary, there was no evidence of Cre-mediated recombination in the brain of the PCT-specific knockout mice, nor any change in expression of other RAS genes. Although, we originally reported low level expression of Cre-recombinase mRNA in the brain of KAP2-iCre transgenic mice, we could not detect evidence of Cre-mediated recombination in the brain of KAP2-iCre X ROSA mice (27).

**Perspectives and Significance**

In summary, our study using two different experimental mouse models has demonstrated that AT1AR in the renal PCT per se are regulators of systemic blood pressure. This finding, confirmed by a recent study also examining the importance of renal PCT AT1A receptors (17), provides additional support for the concept that ANG II regulates blood pressure through its combined actions in multiple sites including the peripheral circulation and local sites of action, such as the renal tubules. This is supported by evidence from our laboratory (13, 23), and collaborative studies with the Navar, Kobori, and Cook laboratories (22, 36) showing that renal-specific expression of the RAS can have effects on systemic arterial pressure without effecting the levels of circulating angiotensin peptides. Further studies are required to elucidate the underlying mechanisms of how PCT AT1AR regulates blood pressure and how it affects AT1 and AT2 receptors in the kidney, and elsewhere, under normal and pathological conditions.

**ACKNOWLEDGMENTS**

The authors thank the University of Iowa Animal Care and Veterinary staff for their assistance in this project. We also thank Norma Sinclair, Patricia Yarolem, and Joanne Schwarting for their technical expertise in generating transgenic mice, and Jaspreet Bassi for technical assistance with receptor autoradiography.
DISCLOSURES

The generous research support of the Roy J. Carver Trust.

Institute (HL-098276). H. Li was supported through a Postdoctoral Fellowship.

Lung, and Blood Institute to C. D. Sigmund (HL-084207, HL-048058, and

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R1076 RENAL PROXIMAL TUBE ANGIOTENSIN AT1A RECEPTORS

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