Angiotensin II regulates growth of the developing papillas ex vivo

Renfang Song, Graeme Preston, Ali Khalili, Samir S. El-Dahr, and Ihor V. Yosypiv

Division of Pediatric Nephrology, Department of Pediatrics, Hypertension, and Renal Center of Excellence, Tulane University Health Sciences Center, New Orleans, Louisiana

Submitted 2 August 2011; accepted in final form 27 January 2012

Song R, Preston G, Khalili A, El-Dahr SS, Yosypiv IV. Angiotensin II regulates growth of the developing papillas ex vivo. Am J Physiol Renal Physiol 302: F1112–F1120, 2012. First published February 1, 2012; doi:10.1152/ajprenal.00435.2011.—We tested the hypothesis that lack of angiotensin (ANG) II production in angiotensinogen- (AGT)-deficient mice or pharmacologic antagonism of ANG II AT1 receptor (AT1R) impairs growth of the developing papillas ex vivo, thus contributing to the hypoplastic renal medulla phenotype observed in AGT- or AT1R-null mice. Papillas were dissected from Hoxb7GFP+/− or AGT+/+, +/-, −/− mouse metanephroi on postnatal day P3 and grown in three-dimensional collagen matrix gels in the presence of media (control), ANG II (10−5 M), or the specific AT1R antagonist candesartan (10−6 M) for 4 h. Percent reduction in papillary length was attenuated in AGT+/− and in AGT+/− compared with AGT+/− (−18.4 ± 1.3 vs. −32.2 ± 1.6%, P < 0.05, −22.8 ± 1.3 vs. −32.2 ± 1.6%, P < 0.05, respectively). ANG II blunted the decrease in papilla length observed in respective media-treated controls in Hoxb7GFP+/− (−1.5 ± 0.3 vs. −10.0 ± 1.4%, P < 0.05) or AGT+/+, +/-, −/− papillas (−12.8 ± 0.7 vs. −18.4 ± 1.3%, P < 0.05, −16.8 ± 1.1 vs. −23 ± 1.2%, P < 0.05, −26.2 ± 1.6 vs. −32.2 ± 1.6%, P < 0.05, respectively). In contrast, percent decrease in the length of Hoxb7GFP+/− papillas in the presence of the AT1R antagonist candesartan was higher compared with control (−24.3 ± 2.1 vs. −10.5 ± 1.8%, P < 0.05). The number of proliferating phospho-histone H3 (phH3)-positive collecting duct cells was lower, whereas the number of caspase 3-positive cells undergoing apoptosis was higher in candesartan- vs. media-treated papillas (phH3: 12 ± 1.4 vs. 21 ± 2.1, P < 0.01; caspase 3: 3.8 ± 0.5 vs. 1.7 ± 0.2, P < 0.01). Using quantitative RT-PCR, we demonstrated that AT1R signaling regulates the expression of genes implicated in morphogenesis of the renal medulla. We conclude that AT1R prevents shrinkage of the developing papillas observed ex vivo control of Wnt7b, FGF7, β-catenin, calcineurin B1, and α3 integrin gene expression, collecting duct proliferation, and survival. Finally, ANG II regulates the expression of genes implicated in morphogenesis of the renal medulla.

MATERIALS AND METHODS

Papillary organ culture. Papillas were dissected from angiotensinogen (AGT)/+−/, +/−, and −/− (Jackson Laboratories, Bar Harbor, ME) or Hoxb7GFP+/− (a kind gift of Dr. F. Costantini, Columbia University, New York) mouse metanephroi on postnatal (P) day P3, suspended in 100 μl of Matrigel (BD Biosciences), and grown for 24 h at 37°C on transwell filters (Corning Costar, 0.5 μm) located on top of DMEM/F12 medium (GIBCO BRL; Fig. 1A). Hoxb7GFP+/− metanephroi express green fluorescent protein (GFP) exclusively in the UB and UB-derived collecting ducts, AGT+/−, +/−, and −/− papillas were grown in the presence of medium with 0.5% FBS (control, n = 6), or medium with 0.5% FBS combined with ANG II (10−5 M, Sigma, n = 6/genotype). Hoxb7GFP+/− papillas were grown in the presence of medium with 0.5% FBS (control, n = 6), or medium with 0.5% FBS combined with ANG II (10−5 M, n = 6), or the specific AT1R antagonist candesartan (Sigma, 10−6 M, n = 6; Fig. 1). We chose to study the effect of 10−5 M ANG II concentrations because high concentrations of ANG II are present in the embryonic kidney (28) and are required to stimulate UB branching (47). Notably, kidney tissue Ang II levels are remarkably higher in the newborn than adult rat kidney (892 ± 91 vs. 103 ± 6 fmol/g) (46). The effect of AGT genotype or drug treatment was studied in paired kidneys obtained

Address for reprint requests and other correspondence: I. V. Yosypiv, Dept. of Pediatrics, SL-37, Tulane Univ. Health Sciences Center, 1430 Tulane Ave., New Orleans, LA 70112 (e-mail: iiosipi@tulane.edu).
Wnt7b, FGF7, epidermal growth factor receptor (EGFR), Hoxb7 reaction was performed three times. Percentage per treatment group were analyzed in triplicates in each run. PCR normalized by that of GAPDH mRNA expression. Three RNA samples were acquired directly from the plates following Matrigel solidification at time of dissection (0 h) and after 24 h of culture via an Olympus IX70 inverted phase-contrast microscope, Olympus MagnaFire FW camera, and processed with Adobe Photoshop 7.0. Percent change in papillary length and surface area, determined by Slide book 4.0 software (Intelligent Imaging Innovations, Denver, CO) at 24 h relatively to tissue 0, was compared between the groups.

Quantitative real-time RT-PCR. Quantitative real-time RT-PCR was utilized to determine whether absence of endogenous ANG II in AGT⁻ /⁻ mice or pharmacologic antagonism of the AT1R with candesartan (10⁻⁶ M, n = 6, or candesartan (10⁻⁵ M, n = 6) for 24 h as described above. In addition, papillas were isolated from AGT⁺ /⁺ and AGT⁻ /⁻ mice on P3. The papillas were fixed in 10% formalin, processed for paraffin embedding, and 4-μm-thick sections were cut. Collecting duct cell proliferation and apoptosis were examined by incubating the sections with anti-phospho-histone H3 (pH3) antibody (Cell Signaling, Danvers, MA; 1:100) or with anti-cleaved caspase-3 antibody (Cell Signaling), respectively. Collecting ducts were visualized with anti-pancytokeratin antibody (Sigma, St. Louis, MO; 1:200). Secondary antibody was Alexa Fluor 594 (Invitrogen, Eugene, OR). The number of pH3- or caspase 3-positive cells was determined in a blinded fashion in four randomly selected sections of each papilla section by fluorescent microscopy and the mean number of pH3- or caspase 3-positive cells per papilla was calculated. All samples were blind-coded for counting. All experiments involving mice were approved by the Tulane Institutional Animal Care and Use Committee. In situ hybridization. Kidneys were dissected from AGT⁺ /⁺ (n = 3) and AGT⁻ /⁻ (n = 3) mice on P3 and processed for in situ hybridization. Preparation of RNA probes and whole mount in situ hybridization were performed according to protocols (http://www.hhmi.ucla.edu/derobertis/protocol_page/-mouse.PDF) established in the De Robertis laboratory. The metanephroi were photographed using an Olympus model SC35 camera mounted on an Olympus model BH-2 microscope, and digital images were captured using Adobe Photoshop software.

Statistical analysis. Data are presented as means ± SE. Differences among the treatment groups in mRNA levels, the number of pH3- or caspase 3-positive cells among the treatment groups in mRNA levels, the number of pH3- or caspase 3-positive cells per papilla was calculated. All samples were blind-coded for counting. All experiments involving mice were approved by the Tulane Institutional Animal Care and Use Committee. Results: Kid, whole P3 kidney.

RESULTS

Effect of ANG II and AT1R antagonist candesartan on papillary growth in the in vitro papillary organ culture. We previously demonstrated that ANG II AT1R mRNA is expressed in the inner medullary collecting duct (IMCD3) cells in vitro and that ANG II, when applied directly to the whole intact E11.5 mouse metanephroi cultured ex vivo, increases the number of UB tips and branch points (21, 47). These findings suggest a potentially novel role for the AT1R in the regulation of UB-derived collecting duct growth. In this study, we utilized...
in vitro papillary organ culture to examine whether hypoplastic papilla observed in AGT- or AT₁R-mutant mice is due to impaired elongation of UB-derived papillary collecting ducts. Notably, P3 papillas grown ex vivo maintain expression of the AT₁R mRNA (Fig. 1B). The basal values of papilla length (μm) were as follows: media: 1,702 ± 154, ANG II: 1,607 ± 115, candesartan: 1,612 ± 210. Papilla length decreased after 24 h of in vitro culture in all treatment groups (Fig. 2). However, percent decrease in the length of the papilla after 24 h of culture compared with respective time 0 was lower in papillas cultured in the presence of ANG II compared with papillas grown in media (control; −1.5 ± 0.3 vs. −10.0 ± 1.4%, P < 0.05; Fig. 2). In contrast, percent decrease in the length of the papilla in the presence of the AT₁R antagonist candesartan was higher compared with control (−24.3 ± 2.1 vs. −10.5 ± 1.8%, P < 0.05). Papillary surface area increased in the presence of ANG II (+12.5 ± 1.9 vs. +1.4 ± 0.4%, P < 0.05) and decreased in the presence of candesartan (−9.7 ± 2.1 vs. −1.4 ± 0.4%, P < 0.05) compared with control. Given that previous studies demonstrated that the administration of the AT₁R antagonist during the first 2 wk of postnatal life leads to papillary atrophy in male but not female rats (37), we next examined whether the effect of exogenous ANG II or candesartan on papilla growth in the in vitro culture is sex dependent. The basal values of papilla length (μm) were as follows: male: media: 1,031 ± 87, ANG II: 1,162 ± 77, candesartan: 1,160 ± 117; female: media: 1,370 ± 79, ANG II: 1,083 ± 67, candesartan: 1,073 ± 105. Percent decrease in the length of the papilla was lower in papillas cultured in the presence of ANG II compared with papillas grown in media in both male (−21 ± 1.0 vs. −32 ± 1.5%, P < 0.05, n = 4) and female (−16 ± 2.3 vs. −28 ± 2.1%, P < 0.05, n = 4) mice. In contrast, percent decrease in the length of the papilla was higher in candesartan-treated male (−44 ± 5.6 vs. −32 ± 1.5%, P < 0.05, n = 4) and female (−39 ± 0.9 vs. −28.5 ± 2.1%, P < 0.05, n = 4) mice compared with control. Percent changes in length in all treatment groups were not different between male and female papillas (media: −32 ± 1.5 vs. −28 ± 2.1%, P = NS; ANG II: −21 ± 1.0 vs. −16 ± 2.3, P = NS; candesartan: −44 ± 5.6 vs. −39 ± 0.9%, P = NS, respectively). The results demonstrate that ANG II, acting via the AT₁R, attenuates the decrease in papillary length that occurs in the media group in the in vitro organ culture in both male and female mice. These findings support the hypothesis that disrupted collecting duct growth may contribute to renal papillary hypoplasia observed in AT₁R-deficient mice in vivo. Lack of the effect of sex on papilla growth in response to ANG II or candesartan in the in vitro system used in our study may be due to absence of systemic estrogens. Given that ANG II AT₂R is expressed in the medullary region during the first week of postnatal life in the mouse and rat (22), the effect of ANG II on papilla growth may be also due, in part, to overstimulation of the AT₂R. Although the

![Fig. 2. Effect of media, ANG II (10⁻⁸ M), or ANG II AT₁R antagonist candesartan (10⁻⁶ M) on papilla growth in vitro. A–F: images of papillas dissected from Hoxb7GFP+ metanephiroi on P3 (time 0) and after 24 h of culture. Collecting ducts are visualized with green color from green fluorescent protein (GFP) fluorescence. Numbers in the bottom left corner of each panel indicate papilla length (μm) at each time point and percent change in papilla length after 24 h of culture compared with respective time 0. G: bar graph showing the effect of media, ANG II, or candesartan on percent change in papilla length.](http://ajprenal.physiology.org/Downloadedfromhttp://ajprenal.physiology.org/by10.1152/ajprenal.00435.2011)
exact role of the AT$_2$R in the regulation of renal medulla formation remains to be determined, occurrence of kidney hypoplasia in a subset of AT$_2$R-mutant mice suggests that such a role is likely (32).

Effect of the absence of endogenous ANG II on papillary growth in the in vitro papillary organ culture. AGT-deficient mice exhibit hydronephrosis and small papilla (29). We hypothesized that papillary hypoplasia in AGT-null mice is due to an intrinsic defect in papillary development due to aberrant elongation of the collecting ducts. To test this hypothesis, we examined elongation of papillas isolated from AGT$^{-/-}$, AGT$^{+/+}$, and AGT$^{+/+}$ metanephi on P3 and grown ex vivo. Papilla length decreased after 24 h of in vitro culture in media-treated papillas in all AGT genotypes (Fig. 3). Observed reduction in papillary length was attenuated in AGT$^{+/+}$ and in AGT$^{-/-}$ compared with AGT$^{-/-}$ (−18.4 ± 1.3 vs. −32.2 ± 1.6%, $P < 0.05$, −22.8 ± 1.3 vs. −32.2 ± 1.6%, $P < 0.05$, respectively) but not in AGT$^{+/+}$ compared with AGT$^{-/-}$ (−18.4 ± 1.3 vs. −22.8 ± 1.3%, $P = NS$) papillas. A decrease in papillary surface area was attenuated in AGT$^{+/+}$ compared with AGT$^{-/-}$ (−17.0 ± 2.3 vs. −25.4 ± 1.5%, $P < 0.05$), but not to AGT$^{+/+}$ (−20.1 ± 2.9%, $P = 0.2$) papillas. We next tested the ability of exogenous ANG II to restore papilla growth in AGT$^{+/+}$ and AGT$^{-/-}$ papillas. ANG II blunted the decrease in papilla length observed in respective media-treated controls in AGT$^{+/+}$, $^{+//-}$, and $^{-/-}$ papillas (−12.8 ± 0.7 vs. −18.4 ± 1.3%, $P < 0.05$, −16.8 ± 1.1 vs. −23 ± 1.2%, $P < 0.05$, −26.2 ± 1.6 vs. −32.2 ± 1.6%, $P < 0.05$, respectively; Fig. 3). Similar changes in papillary surface area were observed in response to ANG II (AGT$^{+/+}$ −9.5 ± 1.7 vs. −17.0 ± 2.3%, $P < 0.05$; AGT$^{+/+}$ −12.0 ± 2.8 vs. −22.1 ± 3.0%, $P < 0.05$; AGT$^{-/-}$ −15.4 ± 2.0 vs. −25.4 ± 1.5%, $P < 0.05$). Percent decrease in papilla length in ANG II-treated papillas was lower in AGT$^{+/+}$ and $^{+/+}$ compared with AGT$^{-/-}$ papillas (−12.8 ± 0.7 vs. −26.2 ± 1.6, $P < 0.05$; −16.8 ± 1.1 vs. −26.2 ± 1.6, $P < 0.05$, respectively), but it did not differ between AGT$^{+/+}$ and $^{+/+}$ (−12.8 ± 0.7 vs. −16.8 ± 1.1, $P = NS$) papillas grown ex vivo. The lowest protective effective of exogenous ANG II observed in AGT$^{-/-}$ papillas may be due to the fact that by the time ANG II was added, some structures already underwent apoptosis and degeneration. Collectively, these findings support the hypothesis that disrupted collecting duct growth may contribute to renal papillary hypoplasia observed in AGT-deficient mice. It is conceivable that renal papilla is destined to regress in the absence of ANG II. This could explain why newborn AGT$^{-/-}$ mice have a papilla but subsequently develop progressively shorter papilla and hydronephrosis because growth of the medulla is not maintained (29).

Effect of AGT genotype and ANG II AT$_1$R antagonist candesartan on collecting duct cell proliferation and apoptosis. To investigate the role of endogenous ANG II in collecting duct cell proliferation and survival during early postnatal development in the mouse, we examined the effect of absence of endogenous ANG II in AGT$^{-/-}$ mice on cell proliferation and apoptosis in kidney papillas isolated on P3. To investigate the

![Fig. 3. Effect of angiotensinogen (AGT) gene dosage on papilla growth in vitro. A–C: images of papillas dissected from AGT$^{+/+}$, $^{+/+}$, $^{-/-}$ metanephi on P3 (time 0) and after 24 h of culture (+24) in the presence of media (control, filled bars) or ANG II (10$^{-3}$ M, open bars). Numbers in the bottom left corner of each panel indicate papilla length ($\mu m$) at each time point and percent change in papilla length after 24 h of culture compared with respective time 0. Scale bar = 500 $\mu m$. D: bar graph showing the effect of media (filled bars) or ANG II (open bars) on percent change in papilla length AGT$^{-/-}$, $^{+/+}$, $^{-/-}$ papillas grown ex vivo.](http://ajprenal.physiology.org/)

AJR Renal Physiol • doi:10.1152/ajprenal.00435.2011 • www.ajprenal.org
role of ANG II AT1R in collecting duct cell proliferation and survival during early postnatal development, we examined the effect of candesartan on cell proliferation and apoptosis in papillas isolated on P3 and grown ex vivo for 24 h. The number of proliferating pH3-positive collecting duct cells delineated by pancytokeratin staining was lower, whereas the number of caspase 3-positive cells undergoing apoptosis was higher in AGT−/− compared with AGT+/+ papillas (P3: 11 ± 0.9 vs. 7 ± 0.8, P < 0.05; caspase 3: 32 ± 3.8 vs. 5 ± 1.1, P < 0.01; Fig. 4). Similar changes in the number of pH3- and caspase 3-positive collecting duct cells were observed in can-

desartan- vs. media-treated papillas (P3: 12 ± 1.4 vs. 21 ± 2.1, P < 0.01; caspase 3: 3.8 ± 0.5 vs. 1.7 ± 0.2, P < 0.01; Fig. 5). These results demonstrate a direct stimulatory effect of endogenous ANG II and its AT1R on collecting duct cell proliferation and survival.

Effect of absence of endogenous ANG II and of AT1R antagonist candesartan on the expression of genes implicated in morphogenesis of the renal medulla. In recent years, murine models have implicated a number of genes, including Wnt7b, FGF7, EGFR, calcineurin, β-catenin, Podl1, β1 and α3 integrins, and p57Kip2, in renal papilla and medulla morphogenesis (4, 9, 13, 25, 36, 48–50). To determine the molecular mechanisms by which ANG II regulates growth of the papilla, we examined the impact of absence of endogenous ANG II in AGT−/− papillas on P3 or pharmacological blockade of ANG II AT1R in P3 papillas grown ex vivo on Wnt7b, β-catenin, FGF7, EGFR, calcineurin B1, β1 and α3 integrin, and p57Kip2 gene expression in vivo by quantitative real-time RT-PCR. Expression of Wnt7b (0.6 ± 0.02 vs. 1.0 ± 0, P < 0.01), β-catenin (0.5 ± 0.1 vs. 1.0 ± 0, P < 0.01), FGF7 (0.4 ± 0.1 vs. 1.0 ± 0, P < 0.01), calcineurin B1 (0.5 ± 0.04 vs. 1.0 ± 0, P < 0.01), and α3 integrin (0.5 ± 0.01 vs. 1.0 ± 0, P < 0.001) mRNA was reduced in AGT−/− compared with AGT+/+ papillas (Fig. 6A). Similar effects were obtained after treatment with candesartan: Wnt7b (0.1 ± 0.03 vs. 1.0 ± 0, P < 0.01), β-catenin (0.04 ± 0.01 vs. 1.0 ± 0, P < 0.01), FGF7 (0.3 ± 0.05 vs. 1.0 ± 0, P < 0.01), calcineurin B1 (0.6 ± 0.08 vs. 1.0 ± 0, P < 0.01), and α3 integrin (0.6 ± 0.01 vs. 1.0 ± 0, P < 0.001) mRNA levels compared with control (Fig. 6B). Absence of AGT or candesartan treatment did not change EGFR, Podl1, β1 integrin, or p57Kip2 mRNA levels compared with control (not shown). Thus, the stimulatory effects of endogenous ANG II on Wnt7b, FGF7, β-catenin, calcineurin B1, and α3 integrin gene expression are mediated, in part, via the AT1R.

Because Wnt7b is essential in papilla formation (48), we examined the spatial expression levels of Wnt7b mRNA in AGT−/− and AGT+/+ metanephoi on P3 by in situ hybridization. Consistent with qPCR findings, expression of Wnt7b was reduced in the medullary collecting ducts of AGT−/− compared with AGT+/+ metanephoi (Fig. 6, C–F). Together, these results suggest that the stimulatory effects of ANG II on papilla growth are mediated by Wnt7b and FGF7, β-catenin, calcineurin B1, or α3 integrin, known positive regulators of papillary morphogenesis (4, 9, 25, 36, 48).

**DISCUSSION**

The underlying mechanisms that regulate normal morphogenesis of the renal medulla and papilla are poorly understood. The critical role of the renin-angiotensin system in the formation of the renal medulla and papilla is evident from occurrence of papillary/medullary hypoplasia and hydronephrosis in AGT−, renin−, angiotensin-converting enzyme (ACE)−, or AT1R-deficient mice (14, 28, 29, 31, 33, 41). Functionally, renin−, ACE−, and AT1R-null animals are polyuric and have a reduced ability to concentrate urine (14, 33, 41). Here, we
identified what we believe to be a novel function for ANG II AT1R signaling in the regulation of papillary morphogenesis. ANG II, acting via the AT1R, prevents a decrease in length of the developing papillas isolated from wild-type or AGT-deficient mice and grown in vitro. Absence of endogenous ANG II in AGT-null mice or inhibition of the AT1R signaling in developing papillas grown ex vivo reduces Wnt7b, /H9252-catenin, FGF7, calcineurin B1, and /H92513 integrin gene expression, decreases proliferation, and induces apoptosis of the collecting duct cells.

Hypoplasia of the renal medulla may result from backward pressure to the renal parenchyma due to congenital structural or functional obstruction of the lower urinary tract (from ureteropelvic junction to the urethra) (6, 9, 27). Recent genetic studies in mice demonstrate that medullary hypoplasia may be due, in part, to an intrinsic defect in medullary morphogenesis normally driven by longitudinal elongation of UB-derived collecting ducts (8). In this regard, UB branching defects may account for medullary hypoplasia observed in mice deficient in bone morphogenic protein receptor, Alk3, AGT, or ANG II AT1R (20, 38, 47). Enhanced collecting duct apoptosis may lead to renal papillary hypoplasia in glypican 3-, B1 and /H92513 integrin-, AT1AR-, or EGFR-null mice (17, 25, 28, 50). Another intrinsic mechanism underlying medullary hypoplasia involves aberrant oriented cell division (OCD) of the collecting duct cells. During normal postnatal development, medullary collecting duct cells divide predominantly along the longitudinal axis leading to collecting duct elongation without a change in diameter (15). Studies in mice demonstrate that collecting duct cell division becomes oriented throughout the kidney on P1 and that 75% of mitotic spindles are oriented within 30% of the longitudinal axis on P5 (23). The importance of the OCD in morphogenesis of the renal medulla and papilla is evident from the observation that targeted inactivation of Wnt7b within the nascent collecting duct epithelium leads to dilation of prospective medullary collecting ducts on E15.5 due to a loss of OCD and results in a failure to form the medulla (48). Wnt7b apparently signals via the canonical Wnt/β-catenin pathway in the renomedullary interstitial cells to direct renal medulla formation (34, 48). Notably, ureter and renal pelvis are comparable between Wnt7b-null and wild-type kidneys (48). Similar to Wnt7b-null mutant renal phenotype, Adams (disintegrin and metalloproteinase with thrombospondin motifs) 1/4-null mice exhibit hypoplastic renal medulla at birth in the absence of structural abnormalities of the lower urinary tract (3). These findings eliminate the possibility of anatomical urinary obstruction leading to papillary atrophy and suggest that hyp-
plastic medulla in Wnt7b and Adamts 1/4 mutants could be due to developmental dysgenesis.

One mechanism proposed to account for the papillary hypoplasia and hydronephrosis in AGT-/-, Renin-/-, ACE-/-, or AT1R-/- deficient mice involves functional urinary tract obstruction. This possibility is supported by the findings in AT1R-null mice that exhibit hypoplastic ureteral smooth muscle layer at birth followed by impaired pelvic peristalsis and an increased intrapelvic pressure at 4 wk thereafter (27). The observed smooth muscle hypoplasia in AT1R-null mice is due to a decrease in the number of proliferating cells in the smooth muscle layer of the pelvis. Of interest, renal medulla forms and is normal at birth in AT1R-null mice but becomes hypoplastic 3 wk after birth. Thus, pelvic contractile forces do not initiate medullary development in these mice.

Several lines of evidence support a potential direct role for ANG II in the regulation of the papilla growth independent of confounding effects of urinary tract obstruction. Kidney tissue ANG II levels are remarkably higher in the newborn than adult rat kidney (892 ± 91 vs. 103 ± 6 fmol/g) (46). AT1R immunoreactivity is present in the UB epithelia on E12.5-E16.5 in the mouse and in the cortical and medullary collecting ducts in the adult rat (19, 22). In addition, cultured IMCD3 cells express AT1R mRNA (21). AT1AR-null mice exhibit decreased expression of the thiazide-sensitive NaCl cotransporter and the \( \beta \)-subunit of the amiloride-sensitive epithelial Na\(^{+}\) channel that are important for salt reabsorption in the collecting tubules (5). ANG II acts via basolateral AT1R to stimulate luminal alkalization in rabbit collecting duct and regulates H\(^{+}\)-ATPase and basolateral K\(^{+}\) channel activities in rat collecting duct (42, 44, 45). It is therefore conceivable that ANG II, acting via the AT1R, may promote functional maturation of collecting duct cells involved in solute transport and acid-base homeostasis. Functionally, renin-, ACE-, and AT1R-
null animals are polyuric and have a reduced ability to concentrate urine (14, 33, 41). Recent studies demonstrate that AT1A receptors in epithelial cells of the collecting duct directly modulate aquaporin-2 levels and contribute to the concentration of the urine in mice (40). Because animals with a longer inner medulla have a higher urine concentration ability (24), it is conceivable that atrophic papilla and inner medulla of the kidney observed in AGT−/−, renin−/−, ACE−/−, and AT1A/R-null mice may contribute to their concentrating defect. An important finding of the present study is that lack of ANG II production in AGT-null mice or pharmacologic antagonism of the AT1R enhances a decrease in length of the developing papillas grown ex vivo. These findings demonstrate that endogenous ANG II, acting via the AT1R, inhibits a decrease in papillary length that occurs in the media group during early postnatal development independent of confounding effects of urinary tract obstruction. Therefore, additional physiological role for ANG II and its AT1R during postnatal papilla development may be to influence the urinary concentrating mechanism through direct effects on medullary collecting duct epithelium. Given that treatment of newborn rats with the specific AT1R antagonist reduces length, volume, and surface area of capillaries in the renal medulla, inhibits organization of the developing vasa recta bundles, and leads to decreased renal blood flow later in life (26), another potential role for AT1R during structural development of the medulla is to promote vasa recta formation.

Increased apoptosis affects the formation of the medulla and papilla in Wnt7b−/−, β1- or α3 integrin-mutant mice (25, 48). Therefore, one mechanism by which aberrant AT1R signaling may disrupt papilla morphogenesis involves increased death and decreased proliferation of the collecting duct cells. The present study demonstrates that inhibition of endogenous AT1R signaling reduces proliferation and enhances apoptosis of the collecting duct cells. We speculate that AT1R-mediated proliferation and survival of collecting duct cells promote longitudinal elongation of the papilla. Our present findings of decreased Wnt7b, FGF7, β-catenin, calcineurin B1, and α3 integrin gene expression in candesartan-treated papillas suggest that the stimulatory effects of the ANG II AT1R on collecting duct elongation are mediated via upregulation of these pathways.

In summary, the present study demonstrates that ANG II AT1R is expressed in the renal papilla during early postnatal development in the mouse. ANG II, acting via the AT1R, prevents shrinkage of the developing papillas in the in vitro organ culture. Aberrant AT1R signaling reduces Wnt7b, β-catenin, FGF7, calcineurin B1, and α3 integrin gene expression, decreases proliferation, and induces apoptosis of the collecting duct cells in developing papillas grown in vitro. These results support the hypothesis that papillary hypoplasia observed in AGT−/− or AT1R-deficient mice is at least partly due to decreased elongation of the collecting ducts, aberrant expression of genes implicated in morphogenesis of the renal medulla, and disrupted collecting duct cell proliferation and survival.

ACKNOWLEDGMENTS

We thank Dr. Frank Costantini (Columbia University Medical Center) for providing Hoxb5−/− mutant mice and Dr. Jing Yu (University of Virginia) for Wnt7b in situ hybridization probe. AGT+/+ C57BL/6/129P2 heterozygous mice (generated by Dr. Oliver Smithies, University of North Carolina) were obtained from Jackson Laboratories (Bar Harbor, ME).

REFERENCES

31. Kriz W.
33. Miyazaki Y, Tsuchida S, Nishimura H, Pope 4th JC, Harris RC.