Novel expression and regulation of the renin-angiotensin system in metanephric organ culture

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Norwood, Victoria F., Marjorie Garmey, Jeffrey Wolford, Robert M. Carey, and R. Ariel Gomez. Novel expression and regulation of the renin-angiotensin system in metanephric organ culture. Am J Physiol Regulatory Integrative Comp Physiol 279: R522–R530, 2000.—To evaluate the presence and regulation of the renin-angiotensin system (RAS) in metanephric organ culture, embryonic day 14 (E14) rat metanephroi were cultured for 6 days. mRNAs for renin and both ANG II receptors (AT1 and AT2) are expressed at E14, and all three genes continue to be expressed in culture. Renin mRNA is localized to developing tubules and ureteral branches in the cultured explants. At E14, renin immunostaining is found in isolated cells scattered within the mesenchyme. As differentiation progresses, renin localizes to the ureteric epithelium, developing tubules and glomeruli. E14 metanephroi contain ANG II, and peptide production persists in culture. Renin activity is present at E14 (6.13 ± 0.61 pg ANG I · kidney⁻¹ · h⁻¹) and in cultured explants (28.84 ± 1.13 pg ANG I · kidney⁻¹ · h⁻¹). Renin activity in explants is increased by ANG II treatment (70.1 ± 6.36 vs. 40.97 ± 1.94 pg ANG I · kidney⁻¹ · h⁻¹ in control). This increase is prevented by AT1 blockade, whereas AT2 antagonism has no effect. These studies document an operational local RAS and a previously undescribed positive-feedback mechanism for renin generation in avascular, cultured developing metanephroi. This novel expression pattern and regulatory mechanism highlight the unique ability of developing renal cells to express an active RAS.

angiotensin receptors; kidney development

IN ADDITION TO THE GENERATION of ANG II by the circulating renin-angiotensin system (RAS), it has been suggested that angiotensins are also produced locally within tissues (10). In the mature kidney, locally produced ANG II contributes to the control of renal blood flow and glomerular filtration rate as well as sodium and water balance (27). ANG II also regulates growth functions in vascular smooth muscle and mesangial cells (32, 37) but the contributions of systemic versus local RASs have not been carefully investigated in growth processes.

The presence of both forms of ANG II receptors (AT1 and AT2) during early metanephric development (34, 41) and the active state of the RAS during kidney development (reviewed in Ref. 18) suggests a role for ANG II in modulating renal morphogenesis and fetal kidney function. In the developing metanephros, RAS components appear before the development of classic functional roles in physiological responses such as systemic blood pressure regulation or renal hemodynamic control (reviewed in Ref. 50), also supporting a role(s) for the renal RAS in kidney morphogenesis. Experimental evidence of the importance of ANG II in renal morphogenesis has been provided by a number of pharmacological inhibition studies demonstrating that blockade of the RAS during pre- and postnatal kidney development results in severe renal dysgenesis (43, 44, 51). More recently, genetically engineered mice lacking angiotensinogen, angiotensin-converting enzyme (ACE), or both AT1a and AT1b receptors have been shown to develop a variety of renal malformations including renal vascular thickening, tubular dilatation, papillary atrophy, and interstitial fibrosis, again illustrating the importance of ANG II in the development and/or maintenance of normal kidney architecture (22, 25, 26, 49).

Although pharmacological studies have clearly shown that renal pathology develops in growing animals in the presence of ACE inhibitors or AT1 antagonists, concerns remain whether these abnormalities are the result of altered placental and/or renal hemodynamics caused by changes in systemic blood pressure in the mother or fetus. Prenatal pharmacological studies are also compromised by the fact that intact drug delivery to the fetus is difficult to document and quantitate. Gene targeting techniques, although exquisitely specific, do not entirely circumvent these issues. Compensatory mechanisms are intact, and potential substitute gene products may allow for normal development to proceed, thereby concealing the function of a deleted gene. Conversely, unmodulated effects of compensatory mechanisms may lead to secondary abnormalities that may be inappropriately attributed to the gene product under investigation. Therefore, a variety of experimental approaches must be considered when dealing with developmental questions.

The present studies were designed to evaluate the presence and regulation of the RAS in metanephric...
organ culture. In this in vitro system devoid of confounding hemodynamic and endocrine influences, undifferentiated metanephric mesenchyme forms avascular glomeruli and tubules. Therefore, documentation of a functional in vitro RAS would provide evidence that a tissue RAS is present in developing kidney tissue and provide an additional important model for pharmacological and molecular approaches to study the mechanisms whereby the RAS controls renal morphogenesis.

MATERIALS AND METHODS

Animals. Timed-pregnant Sprague-Dawley rats were purchased from Hilltop Farms (Scottsdale, PA) with time zero [embryonic (E) day 0 (E0)] designated as the earliest possible time of mating. The window for conception was limited to 6 h to maintain reproducible developmental staging. After induction of anesthesia with pentobarbital sodium, the kidneys from E14 fetuses were microdissected from surrounding tissue at 4°C in 1:1 DMEM and Ham’s F12. The accuracy of gestational age was documented by comparison with published embryologic anatomy (20). All procedures were performed with the approval of the University of Virginia Animal Research Committee.

Metanephric organ culture. After methods similar to Avner et al. (3), dissected metanephroi were placed on transparent cell culture inserts (Cyclopore membranes (9-mm pore size), Becton Dickinson, Lincoln Park, NJ) in a six-well culture plate. The serum-free media, DMEM-F12, was supplemented with HEPES (10 mM), NaHCO3 (1.1 mg/ml), penicillin G (50 units/ml), mycostatin (50 units/ml), gentamicin (10 mg/ml), insulin/transferrin/selenium mixture (5 mg/ml as insulin), prostaglandin E1 (25 ng/ml), and triiodothyronine (32 pg/ml). All media supplements were obtained from Sigma (St. Louis, MO). The samples were cultured at 37°C in humidified 95% air-5% CO2 for 6 days. For studies of receptor-mediated regulation of renin activity, explants were cultured in the presence or absence of ANG II (10^-6 M), losartan (10^-6 M), and adult kidneys (n = 3) was extracted using the method of Chomczynski (8). After RNA spectrophotometric quantitation, RT-PCR was carried out using standard techniques. First strand cDNA synthesis was performed using 0.5 μg of sample RNA, 10 units M-MuLV RT (Boehringer Mannheim, Indianapolis, IN), 2.5 mM dNTPS, and 50 nM downstream primer in a 20-μl reaction at 42°C for 45 min. The synthetic primers (Operon Technologies, Alameda, CA) used for renin amplification, 5’-ATGCCCTCTCTGGGACACTTGT-3’ (upstream) and 5’-GATCTGGGAGCCAGCATGA-3’ (downstream), were designed to yield a 638-bp fragment of the full length rat renin cDNA described by Burnham et al. (5). Amplification was carried out by thermal cycling (denaturation at 94°C and extension at 72°C) for 30–35 cycles with a primer annealing temperature of 55°C using a DNA thermal cycler (Perkin Elmer Cetus, Foster City, CA). The primers for AT1 amplification were designed to yield a 638-bp fragment corresponding to nucleotides 253–890 of the full length cDNA described by Murphy et al. (29). These primers, 5’-CTCAACCAGAAAAACAAAA-3’ (upstream) and 5’-GGAACAGGAAGCCCCAGATA-3’ (downstream), detect both AT1α and AT1β isoforms. AT1 amplification was carried out by thermal cycling (denaturation at 95°C and extension at 72°C) for 30–35 cycles with a primer annealing temperature of 55°C. Primers used for AT2 amplification, 5’-AAACACTGCGAACTAACAG-3’ (upstream) and 5’-CAAGGGAGGACTACATAAG-3’ (downstream), were designed to yield a 551-bp fragment of the full-length cDNA described by Dzaa et al. (28). AT2 amplification was carried out by thermal cycling for 30–35 cycles using an annealing temperature of 55°C. Total RNA (0.5 μg) was used in each reaction, and the entire series of reactions was repeated in triplicate. Detection of PCR products was obtained by agarose gel electrophoresis and ethidium bromide staining. Product identity was confirmed for all fragments using direct DNA sequencing.

Negative controls for the reaction included omission of RT and omission of Taq polymerase. Adult liver RNA was used as a negative control for AT2. Positive controls included the full-length cDNA of interest and total RNA from tissues expected to contain the message (adult kidney for AT1 and renin and adult adrenal for AT2).

Fig. 1. RT-PCR for renin. The ethidium-stained agarose gel shows the expected 560-bp products of amplification using primers specific for rat renin. Ad K, adult kidney is positive control; E14, prevascular embryonic day 14 metanephroi; X6, explants cultured in serum-free media for 6 days.

Fig. 2. RT-PCR for AT1 and AT2 receptors in E14 kidneys and cultured explants (X6). The ethidium-stained agarose gel shows the 638-bp product of AT1 amplification and the 551-bp product of AT2 amplification. Lane 1: adult kidney + AT1 primers. Lane 2: E14 + AT1 primers omitting RT. Lane 3: E14 + AT1 primers. Lane 4: 1-kb DNA ladder. Lane 5: E14 + AT2 primers. Lane 6: E14 + AT2 primers omitting RT. Lane 7: X6 + AT1 primers. Lane 8: X6 + AT1 primers omitting RT. Lane 9: 1-kb DNA ladder. Lane 10: X6 + AT2 primers. Lane 11: X6 + AT2 primers omitting RT. Lane 12: adult kidney + AT2 primers.

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ANG II content. E14 metanephroi (n = 28 per pool) and X6 explants (n = 12–15/pool) were homogenized in ice-cold methanol and assayed as previously described (42). Briefly, samples were acidified (1:1) with 0.6% trifluoroacetic acid and extracted over octyldecylsilane cartridges (Sep-Pak, Waters Associates).

After evaporation, the dried extracts were reconstituted in 0.01 M phosphate buffer (pH 7.4) and assayed by radioimmunoassay using 125I-labeled ANG II (New England Nuclear) and a rabbit anti-ANG II antibody (Peninsula Laboratories). This antibody cross-reacts with ANG III and IV but not ANG I. The sensitivity of this assay (concentration giving 90% maximal inhibition [IC90]) is 0.93 pg. Three separate pools of metanephroi and explants were assayed.

In situ hybridization for renin mRNA. X6 (n = 6) metanephroi were washed in cold PBS and fixed in 3% buffered paraformaldehyde at 4°C followed by sucrose protection, as previously described (17). Cryosections (10 μm) were post-fixed for 5 min in cold paraformaldehyde and acetylated using 0.1 M triethanolamine followed by 0.25% acetic anhydride. Hybridization was carried out overnight at 42°C in buffer containing 50% formamide, 25% 20× sodium chloride-sodium citrate (SSC), 1% Denhardt’s solution, 0.25 g% dextran sulfate, 250 μg/ml herring sperm DNA, and 250 μg/ml yeast tRNA. Digoxigenin-labeled cRNA sense and antisense riboprobes were generated from a full-length rat renin cDNA, kindly provided by Dr. Kevin Lynch, using the Genius system (Boehringer Mannheim, Indianapolis, IN) following manufacturer’s instructions and T7 and SP6 RNA polymerases. The full-length probe was hydrolyzed using alkaline hydrolysis to yield ~200-bp fragments. Probe concentrations were determined by color detection and used in hybridization reactions at a final concentration of 50 ng/ml. After hybridization, sections were treated with 40 μg/ml RNase A and then washed in 1× SSC (3 × 5 min), 0.2× SSC + 25% formamide at 50°C (2 × 15 min), and 0.5× SSC (1 × 5 min). Immunological detection of the digoxigenin-labeled probes was performed using an alkaline phosphatase conjugated anti-digoxigenin antibody, X-phosphate, and nitroblue tetrazolium per manufacturer’s instructions.

Renin immunohistochemistry. Tissues for morphological analysis and immunohistochemistry were fixed in Bouin’s fixative and embedded in paraffin. Seven-micrometer sections were stained for renin using a previously characterized polyclonal goat anti-rat renin antibody (gift of T. Inagami, Vanderbilt University, Nashville, TN) (31), avidin-biotin-peroxidase (Vectastain ABC kit, Vector Laboratories, Burlingame, CA), and methods described previously (17). Negative controls included omission of primary and secondary antibodies.

Tissue renin activity. E14 metanephroi (n = 28) and X6 explants (n = 12–15) were homogenized and sonicated in lysis buffer containing 150 mM NaCl, 0.5 mM EDTA, 25 mM HEPES, 1% Triton X, 0.5% deoxycholic acid, and 0.5 mM phenylmethylsulfonyl fluoride. Renin activity was determined using ANG I generation in the presence of excess substrate followed by ANG I radioimmunoassay as previ-
A

B

C

ous described (23). Three to seven separate pools of meta-
nephroi and explants were assayed.

Statistics. The data are presented as means ± SE. Tissue renin activities were compared using t-test or ANOVA with Bonferroni correction when appropriate. Tissue ANG II levels were compared by unpaired t-test. Values were considered significant at $P < 0.05$.

RESULTS

RT-PCR for renin $AT_1$ and $AT_2$. Total RNA isolated from E14 metanephroi and explants cultured for 6 days were assayed by RT-PCR for the presence of mRNA for renin and the two angiotensin receptors $AT_1$ and $AT_2$. Figure 1 shows the ethidium-stained gel containing renin products. The expected 560-bp product is seen both at E14 and following culture (X6). Omission of RT did not lead to amplification of a product (data not shown), documenting that the bands are due to the amplification of RNA, not genomic DNA. These results indicate that at the prevascular stage of metanephric development, there are cells within the developing kidney that already express the renin gene. In addition, the expression of the renin message is maintained in the avascular in vitro environment during glomerular and tubular differentiation.

Figure 2 shows the RT-PCR products for $AT_1$ and $AT_2$ receptors in both E14 metanephroi and cultured explants. Expression of both receptor subtypes is present at E14 and is maintained in culture mirroring the in vivo pattern of gene expression. As expected, $AT_1$ was expressed in the adult rat kidney, but $AT_2$ was not. Likewise, no amplification of either product was seen in the absence of RT.

ANG II content. At E14, metanephroi contain 1.02 ± 0.16 pg ANG II/explant (equal to 1.07 fmol/explant, 15.7 pmol/g wet weight, and 0.160 fg/μg protein). After 6 days in culture, ANG II increases to 7.08 ± 0.63 pg ANG II/explant ($P < 0.001$; equal to 7.43 fmol/explant, 19.46 pmol/g wet weight, and 0.360 fg/μg protein). These results suggest a persistent and increasing presence of endogenous ANG II during in vitro growth.

In situ hybridization for renin mRNA. Cultured explants and adult kidneys hybridized with antisense and sense riboprobes for renin are shown in Fig. 3. The purple staining indicative of hybridization is clearly seen in developing tubules and ureteral branches of the explant (Fig. 3A). Glomeruli and undifferentiated mesenchyme do not contain detectable renin mRNA. The sense probe shows no specific staining, confirming the specificity of the antisense probe (Fig. 3B). As an additional control, the same antisense probe was hybridized with a section of similarly prepared adult kidney

Fig. 4. Renin immunohistochemistry in E14 metanephroi. A: low power (×62). B: high power (×125). Brown peroxidase staining localizes renin to scattered individual cells within the undifferentiated mesenchyme and adjacent to forming epithelial structures. Renin-positive cells are relatively large, contain large amounts of granular cytoplasm and small nuclei, and have no distinct cell-cell attachments. The ureteric epithelium (U) and developing nephron structures are negative. C: negative control using nonimmune serum (×62). No brown staining is present.
tissue (Fig. 3C). The specific juxtaglomerular localization classically ascribed to renin is easily seen and also confirms the sensitivity and specificity of the riboprobe. These results indicate that renin gene expression in avascular kidney explants is maintained by tubules and ureteric bud derivatives.

Renin immunohistochemistry. The E14 metanephros is composed of undifferentiated mesenchyme, branching ureteric columnar epithelium, and nephron precursors undergoing condensation and mesenchymal-to-epithelial transformation. At this stage, renin immunostaining is found in isolated cells scattered within the undifferentiated mesenchyme (Fig. 4). Compared with neighboring mesenchymal and epithelial cells, renin-positive cells have relatively small nuclei, plentiful cytosol, and no obvious cell attachments. At E14, the ureteric epithelium is clearly negative. During 6 days in serum-free culture, explants undergo differentiation with development of avascular glomeruli, tubules, and interstitial areas. Renin protein expression shifts from the isolated cells seen at E14 into the ureteric epithelium, developing tubules and glomeruli (Fig. 5). The urinary pole of Bowman’s capsule and early proximal tubule contain renin protein, whereas condensed mesenchyme, undifferentiated mesenchyme and interstitial compartments do not. C: negative control using non-immune serum (×62). No brown staining is present. D: in vivo time control for X6 explants showing the expected large vessel expression of renin in a kidney from an E20 rat fetus. No glomerular or tubular renin expression is noted.

To assess the regulation of renin activity in cultured explants by ANG II and its receptors, explants were treated for the entire culture period with ANG II, losartan, PD-123319, or combinations of these (all at 10^-6 M). Measurements of protein content at X6 revealed no differences in growth of the cultured explants under any of the experimental conditions (data not shown). Figure 6 shows that renin activity is increased by ANG II (70.1 ± 6.36 vs. 40.97 ± 1.94 pg ANG I · kidney^-1 · h^-1, P < 0.001) and that this increase is completely blocked by the AT1 antagonist losartan. The AT2 antagonist PD-123319 had no effect on the ANG II-mediated increase in renin activity, and neither inhibitor altered baseline renin activity. These results indicate that the increase in renin activity caused by ANG II is mediated through an AT1-receptor.

Table 1. Renin activity in E14 and cultured metanephroi

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<th>E14 (31–38 explants each)</th>
<th>X6 (12 explants each)</th>
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<tr>
<td>pg ANG I · kidney^-1 · h^-1</td>
<td>6.13 ± 0.61</td>
<td>28.44 ± 1.13^*</td>
</tr>
<tr>
<td>pg ANG I · µg protein^-1 · kidney^-1 · h^-1</td>
<td>1.16 ± 0.20</td>
<td>1.09 ± 0.10</td>
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Values are means ± SE; n, pools. Renin activity was measured by production of ANG I from excess substrate. Active renin content per kidney increased during in vitro culture [embryonic day 14 (E14) vs. explants after 6 days in culture (X6), ^P < 0.001] commensurate with increased explant protein content during growth.
mechanism and that $AT_2$ signaling does not affect renin activity. In addition, the lack of suppression of renal activity by either antagonist in the absence of exogenous ANG II suggests that the inherent production of renin by metanephric explants occurs independently of endogenous ANG II and ANG II receptor control.

**DISCUSSION**

The present study demonstrates a novel expression pattern and regulatory mechanism for renin gene expression in the embryonic kidney in vitro. These results document the plasticity of renin gene expression and highlight the unique ability of differentiating renal cells to maintain generation of ANG II in the absence of normal vascular sources.

During in vivo renal development in rats, renal renin mRNA and protein are expressed exclusively in the vasculature, beginning at E17 in Wistar (40) and Sprague-Dawley (36) strains and at E19 in Wistar-Kyoto (17). The pattern of expression shows a shift in renin distribution from larger arteries in prenatal life to the classical juxtaglomerular (JG) localization in adulthood. The presence of renal angiotensinogen mRNA has been described as early as E17 (16), and renal ACE is present by E16 (24). $AT_1$ and $AT_2$ angiotensin receptors are present at E14 with a predominance of $AT_2$ expression (34). By the time of birth, $AT_1$ expression has increased significantly, and $AT_2$ down-regulates rapidly in the first two postnatal weeks (9, 34). Bensoussan et al. (4) have previously proposed that metanephric organ culture is an appropriate model for the study of the ontogenic changes in angiotensin receptors. With the use of radioligand binding autoradiography and Northern blot hybridization analysis, they showed that both $AT_1$ and $AT_2$ receptors were present in the E14 rat metanephros with a predominance of $AT_2$ binding (94%). After 6 or 13 days in culture, $AT_1$-receptor expression increased, mimicking the in vivo shift in receptor expression (4). Although we did not attempt to quantitate the changes in ANG II receptor expression during culture, the current study confirms that both receptor subtypes are expressed before renal vascularization (E14) and that expression is maintained for a minimum of 6 days in the in vitro environment. The present study shows that renin, ANG II, and both types of angiotensin receptors are present in the prevascular metanephros at its inception (E14) and suggests potential morphogenetic and growth functions for the system. In addition, the components of this tissue RAS continue to be expressed and act in a regulated fashion in the isolated, avascular conditions of metanephric organ culture. Although the functions of this isolated RAS have not yet been determined, the data show that the isolated kidney can and does produce renin and ANG II in the absence of vascular supply. It is interesting to speculate that this RAS may provide the ANG II necessary for the developmental process before the development of vessels.

The current study shows that renin is expressed as early as E14 in the rat metanephros, earlier than previously reported. In this study, renin mRNA was detected by RT-PCR, the most sensitive means of detecting mRNA, whereas prior studies have studied older fetuses and relied on Northern blot hybridization or in situ hybridization. Immunocytochemical evidence of renin within early metanephros has been previously described only within the large vessels, not within the scattered undifferentiated cells seen in this study. Although most studies have evaluated older fetuses, it is possible that, given their scarcity and the relatively low staining density compared with JG cells, these occasional cells may have been overlooked in previous studies. It is interesting to speculate that the renin-positive cells at E14 may be the vascular precursors for the cells that form the renin-positive afferent vasculature and ultimately the JG cell during normal development.

This report of renin mRNA and protein in developing glomeruli and ureteric bud derivatives is a unique finding suggesting novel regulatory mechanisms. Recently, Arend et al. (2) reported renin mRNA production by both mesenchymal and ureteric bud derivatives in chimeric metanephros. In our study, renin mRNA was not detected by in situ hybridization in developing glomeruli, although the protein was seen by immunohis-
tochemistry. This discrepancy may be due to the relative insensitivity of in situ hybridization for low-quantity transcripts, or perhaps renin protein is taken up by the developing glomerulus but not made there. Currently, the function of renin within tubules, branching ureteric bud, and bud derivatives is not clear. Renin appears to be involved in vascular branching morphogenesis (39) and could perhaps play a role in the branching of the ureteric bud derivatives as well. Clearly, these cells are capable of synthesizing renin gene under the avascular culture conditions. Because renin mRNA and protein have been found in mature glomeruli and tubular epithelial cells under pathological conditions (discussed below), it is reasonable to consider that a variety of immature renal precursor cells may be capable of renin production during the differentiation process. In fact, this system may be induced to provide the ANG II necessary for growth.

The finding of renin within glomeruli and tubules in cultured explants, although unexpected, is not without precedence in other models. In vivo, the proximal tubule has all of the components needed to generate ANG II (6), but it has been argued that proximal tubular renin is taken up from the glomerular filtrate (47). However, other authors have shown that under certain conditions, renin may be synthesized by cells not usually thought to account for significant renin production. Renin mRNA and protein have been found within glomerular endothelial and mesangial cells and adrenal vascular smooth muscle in a child with a hypertensive Barter’s-like syndrome (38) and in microdissected rat proximal tubules (46). Likewise, renin mRNA and protein have been found in cultured adult rat mesangial cells (13), embryonic pig proximal tubules (14), rat and rabbit proximal tubule cell lines (7, 21, 45), as well as a large variety of nephron cells after 24-h ureteral obstruction in the adult rat (35). Renin has also been found in the cystic epithelium of human kidneys with autosomal dominant polycystic kidney disease (48), suggesting that a variety of stimuli may induce the tubular epithelium to produce renin. Interestingly, there exists a temperature-sensitive transformed rat proximal tubule cell line that contains mRNA and protein for renin, angiotensinogen, ACE, and the AT1 receptor, suggesting that in some conditions, a complete and functional RAS may be present within a single renal cell (45).

The presence of biochemically active renin in avascular kidney organ culture is a previously undescribed phenomenon. The renin activity values obtained in the present study (~1–2 ng ANG I · h⁻¹ · mg protein⁻¹) are significantly lower than those reported in adult rodent kidneys (~10–5,000 ng ANG I · h⁻¹ · mg protein⁻¹) (1, 33). However, the renin activities in cultured explants are similar to those reported in left ventricle (10), testis, small intestine, liver, spleen, and aorta (12) and rat and rabbit proximal tubule cells (21). Because the in vitro culture system does not result in the formation of differentiated JG cells, it seems logical that the high levels seen in mature kidney or in cultured JG cells (total renin ~9 μg ANG I · h⁻¹ · mg protein⁻¹) (11) are not achieved in this culture system. It is also possible that a portion of the immunoreactive renin seen in explants is actually inactive prorenin that does not contribute to the constitutive generation of angiotensin peptides. Whether or not the renin present in explants is actively secreted in a regulated fashion remains to be determined.

This is the first report to document the presence of ANG II within kidney tissue during early metanephric development. In addition, this study shows that isolated, avascular explants growing in serum-free culture continue to generate angiotensin peptides. In adult kidney tissue, ANG II contents range from 0.1 to 2 pmol/g kidney, whereas proximal tubular fluid ANG II levels are even higher (8.1 ± 1.6 pmol/ml) (30). E14 and cultured metanephroi therefore contain high levels of ANG II (~15–20 pmol/g wet weight) compared with adult tissues. This could be due to relative inactivity of angiotensinases allowing maintenance of high peptide levels. An additional potential reason for relatively high ANG II levels seen in these experiments may be cross-reactivity of ANG II degradation peptides (primarily ANG III and ANG IV) in the RIA. Because the assay does not discriminate between these peptides, degradation products could be detected. Further studies using HPLC techniques could potentially determine the relative proportions of angiotensin peptides within the developing kidney.

The most common mechanism by which ANG II regulates renin activity is by a negative-feedback mechanism, usually an AT1-mediated suppression of renin production and secretion (reviewed in Ref. 19). The present study, however, suggests that ANG II may stimulate renin activity through an AT1-receptor mechanism. AT2 antagonism had no effect on renin activity. Although uncommon, positive feedback loops for the RAS have been reported. ANG II, acting through AT1 receptors, enhances renin gene expression in hepatic nuclei (15). In addition, ipsilateral renin, AT1 receptors, and local ANG II levels are all elevated in unilateral ureteral obstruction (52), suggesting that the usual negative feedback of ANG II on renin activity can be disrupted under some conditions. In this in vitro model, baseline renin activity was not altered by either AT1 or AT2 blockade, suggesting a constitutive production of active renin by cultured explants that is independent of ANG II regulation. Because the majority of renin in this model is generated and contained within tubular elements, it is perhaps most appropriate to consider renin regulatory mechanisms present in non-vascular tissues. Henrich et al. (21) have shown that rabbit proximal tubules produce and secrete renin in both constitutive and regulated processes. Renin activity is enhanced by isoproterenol, but ambient calcium concentration does not affect angiotensin generation, suggesting that the regulated components of renin activity in these cells are different from those seen in JG cells (21). Chen et al. (7) have also concluded that rat proximal tubule renin is regulated differently from vascular and glomerular renin because its production is not altered by ACE inhibition. In a recent report,
Tank et al. (46) suggest that failure to suppress proximal tubule renin production in response to sodium loading may contribute to the generation of salt-sensitive hypertension in the Dahl/Rapp rat. It is interesting to speculate that early nephron tissue may constitutively express renin as a mechanism to maintain ANG II production before the differentiation of vascular tissue and JG cells capable of expressing the renin gene at high levels. The potential function for the positive feedback loop seen in metanephric organ culture remains unclear.

In summary, our studies document that renin, ANG II, and both angiotensin receptors are present in the prevascular E14 metanephros and that their expression is maintained for at least 6 days in serum-free organ culture. In this avascular model, renin mRNA and protein are produced by tubules and cells of ureteric bud lineage, suggesting that renal cells not usually thought to make renin can be induced to do so in the absence of differentiating JG cells. Regulated renin activity in this model is stimulated by the addition of exogenous ANG II through an AT1 receptor-mediated mechanism, whereas constitutive renin activity is not controlled through ANG II receptors. The findings in this study are distinctly different from the classical ontogeny and developmental regulation of the in vivo RAS. However, in the absence of normal vasculature and JG cell differentiation, it seems clear that developing renal tissue can, and does, generate ANG II. Clearly, a local RAS exists in metanephric tissue. The novel expression patterns and regulatory controls exhibited by this system underscore the plasticity of renal cells and may provide insights into heretofore unexpected actions of the RAS in renal development.

Perspectives

The finding of renin production and activity in avascular kidney explants suggests that a number of differentiating renal cell lineages have the capacity to participate in ANG II generation early in nephrogenesis. As renal development proceeds, the majority of ANG II production results from the regulated action of renin secreted by differentiated JG and vascular cells. At present, one can merely speculate whether the prevascular tissue RAS described in these experiments is absent during normal renal development, overshadowed by the more highly expressed vascular/JG system or actively suppressed by the classic pathway. Likewise, the physiological significance of the metanephric RAS must for now remain speculative. It is possible that a number of developing cell types develop the machinery for ANG II generation during metanephric differentiation and that the persistence and/or loss of this capacity are merely markers for the normally differentiated phenotype that are altered in the culture environment. However, it is intriguing to speculate that constitutive, non-AT1-regulated renin production and a positive-feedback loop for ANG II-/AT1-mediated renin production may be “safety valves” for maintenance of ANG II production under conditions of vascular insufficiency. ANG II is certainly necessary for normal renal vascular development, and one might speculate that site-specific production of renin and/or ANG II by nonvascular cells may guide appropriate vascular formation within the developing metanephros. In addition, the importance of ANG II in the control of intracellular fluid and electrolyte content and homeostasis during early tubular differentiation is unknown. One might hypothesize that intracellular or extracellular generation of ANG II may be necessary to maintain the appropriate milieu for growth, proliferation, differentiation, and architectural organization. In that case, the constitutively expressed RAS described in this paper is appropriately poised to maintain needed ANG II production during the complex rearrangements and functional changes characteristic of organogenesis.

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REFERENCES


