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# The intrarenal renin-angiotensin system in autosomal dominant polycystic kidney disease

**Mahmoud Loghman-Adham, Carlos E. Soto, Tadashi Inagami, and Lisa Cassis**

*Department of Pediatrics and Pediatric Research Institute, Saint Louis University, St. Louis, Missouri 07920*

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**Loghman-Adham, Mahmoud, Carlos E. Soto, Tadashi Inagami, and Lisa Cassis.** The intrarenal renin-angiotensin system in autosomal dominant polycystic kidney disease. *Am J Physiol Renal Physiol* 287: F775–F788, 2004. First published June 8, 2004; 10.1152/ajprenal.00370.2003.—Hypertension is a common complication of autosomal dominant polycystic kidney disease (ADPKD), often present before the onset of renal failure. A role for the renin-angiotensin system (RAS) has been proposed, but studies of systemic RAS have failed to show a correlation between plasma renin activity and blood pressure in ADPKD. Ectopic renin expression by cyst epithelium was first reported in 1992 (Torres VE, Donovan KA, Sicli G, Holley KE, Thibodeau ST, Carretero OA, Inagami T, McAteer JA, and Johnson CM. *Kidney Int* 42: 364–373, 1992). It is not known, however, whether other RAS components are also expressed by cysts in ADPKD. We show that, in addition to renin, angiotensinogen (AGT) is produced by some cysts and dilated tubules. Angiotensin-converting enzyme, ANG II type 1 receptor, and ANG II peptide are also present within cysts and in many tubules; and some cyst fluids contain high ANG II concentrations. Additionally, cyst-derived cells in culture continue to express the components of the RAS at both the protein and mRNA levels. We further show that renin is expressed primarily in cysts of distal tubule origin and in cyst-derived cells with distal tubule characteristics, whereas AGT is expressed primarily in cysts of proximal tubule origin and in cyst-derived cells with proximal tubule characteristics. Renin production by cyst-derived cells appears to be regulated by extracellular $Na^+$ concentration. Based on these observations, we propose a model of an autocrine/paracrine RAS in polycystic kidney disease, whereby overactivity of the intrarenal system results in sustained increases in intratubular ANG II concentrations.

hypertension; cyst epithelium; polycystic kidney disease; pressure-natriuresis; renin

AUTOSOMAL DOMINANT POLYCYSTIC kidney disease (ADPKD) is a common genetic disorder resulting in the formation of cystic dilatation of renal tubules, leading to a gradual destruction of renal parenchyma and renal failure in half of the patients by age 60 (59). Mutations in two genes, *PKD1* and *PKD2*, that encode membrane-associated proteins polycystin-1 and -2 account for almost all cases of ADPKD, with *PKD1* mutations accounting for ~85% of the cases (23, 59). In ADPKD, cysts may originate from any nephron segment, including proximal and distal tubules or the collecting ducts (1, 10).

Hypertension is observed in over half of the patients with ADPKD, often present before the onset of renal insufficiency (3, 8, 35). It is a major factor in the progression toward end-stage renal disease (31). The mechanisms leading to hypertension in ADPKD are not well understood. Hypertension appears to be associated with larger kidney size, which may reflect a larger number of cysts (45). Involvement of the renin-angiotensin system (RAS) has been postulated, but no consistent relationship has been found between blood pressure and plasma renin activity or plasma aldosterone concentrations (3, 7, 14, 45). Only indirect evidence is available to support involvement of the RAS in blood pressure control in ADPKD (3, 7, 14). For example, the administration of captopril, a converting-enzyme inhibitor, results in a significantly greater rise in plasma renin activity in hypertensive compared with normotensive ADPKD patients, suggesting overactivity of the RAS (3). Although it is difficult to correlate the increased activity of the systemic RAS with hypertension in ADPKD, there is some evidence to support the overactivity of the intrarenal RAS in this condition. Torres et al. (52) reported strong renin immunostaining in dilated tubules and cysts in ADPKD kidneys. Furthermore, they showed that cyst-derived epithelial cells in culture contain immunostainable renin and express renin mRNA, suggesting local renin synthesis (52). These studies showed that, in ADPKD, cyst epithelium could produce renin but did not explore whether other RAS components are also present in ADPKD cysts and whether increased tubulocystic renin may lead to increased ANG II production.

In the present study, we have confirmed the observations of Torres et al. (52) and discovered that, in addition to renin, angiotensinogen (AGT), angiotensin-converting enzyme (ACE), ANG II receptor, and ANG II peptide are also present in cysts and in dilated tubules in ADPKD kidneys. Based on these findings, we hypothesize that ectopic renin and AGT production by cyst epithelium could result in increased formation of ANG I, followed by increased ANG II production. High intratubular ANG II concentrations could cause increased sodium and water reabsorption by the functioning tubules, which over time could result in hypertension. We propose a possible model of autocrine/paracrine intrarenal RAS to account for salt and water retention and hypertension observed in polycystic kidney disease.

## METHODS

*Immunohistochemistry of tissue sections.* Polycystic kidneys were surgical specimens shipped on ice from many U.S. sites and processed within 24 h of nephrectomy. The main reason for the nephrectomy was preparation for a kidney transplant. Kidney sections were fixed in 10% buffered formalin, pH 7.4, and paraffin embedded. Sections (4 μm) were used for immunohistochemistry. The sections were deparaffinized in HemoDe (Fisher Scientific, Pittsburgh, PA) and then rehydrated in graded alcohols. Antigen retrieval was performed with 0.1 M citrate buffer, pH 6.0, at 60°C for 30 min. The slides were rinsed two times with PBS, followed by the addition of 0.6% $H_2O_2$ in

Address for reprint requests and other correspondence: M. Loghman-Adham, 26 Huntington Rd., Basking Ridge, NJ 07920 (E-mail: mloghman@att.net).

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Table 1. *Information on antibodies used*

| Antigenic Protein | Host or Origin | Type | Source | Useful Dilutions | |
|---|---|---|---|---|---|
| | | | | Paraffin tissue sections | Paraformaldehyde-fixed cells |
| Purified human kidney renin | Rabbit | Polyclonal antiserum | T. Inagami | 1:2,000–1:8,000 | 1:100–1:500 |
| Human renin 14-mer peptide | Rabbit | Polyclonal affinity purified | M. Loghman-Adham | 1:500–1:1,000 | 1:50–1:200 |
| Human angiotensinogen | Rabbit | Polyclonal antiserum | D. Tewksbury | 1:4,000–1:32,000 | 1:500 |
| ACE | Mouse | Monoclonal | Chemicon | 1:100–1:500 | 1:100–1:200 |
| ANG II ($AT_1$) receptor | Rabbit | Polyclonal affinity purified | Santa Cruz BT | 1:500–1:1,000 | 1:250 |
| ANG I/ANG II peptides | Mouse | Monoclonal | Santa Cruz BT | 1:500–1:1,000 | 1:250 |
| ANG II peptide | Rabbit | Polyclonal affinity purified | Peninsula Labs | 1:250–1:500 | 1:50–1:100 |
| Aminopeptidase N (CD-13) | Mouse | Monoclonal | Santa Cruz BT | ND | 1:250 |

ACE, angiotensin-converting enzyme; $AT_1$, ANG II type 1 receptor; ND, not done; BT, Biotechnology.

20% methanol for 20 min at room temperature to block endogenous peroxidase. The slides were washed three times with PBS and then blocked with normal horse serum for 20 min at room temperature. The primary antibodies were added at the dilutions indicated in Table 1. The slides were incubated either for 1 h at room temperature or overnight at 4°C and then washed three times with PBS-0.1% Tween 20, followed by the addition of the second biotinylated antibody and incubated for 30 min at room temperature.

For lectin-binding studies, biotinylated *Lotus tetragonolobus* (LTA) and *Arachis hypogaea* (PNA) lectins were used directly at this stage. LTA is a marker of proximal tubules, and PNA is a marker of distal and collecting tubules. Tissue sections were washed three times with PBS followed by the addition of one drop of the ABC reagent (Vectastain Elite kit; Vector Laboratories, Burlingame, CA) and incubated at room temperature for 30 min. The slides were washed three times with PBS, followed by the addition of peroxidase substrate for 6–10 min. They were rinsed in distilled water, counterstained with hematoxylin (Gill No 3; Sigma Diagnostics) for 60–90 s, washed extensively in running water, and mounted. They were viewed with a Zeiss Axioplan microscope and photographed with Kodak Ektachrome 64T film.

*Culture of cyst-derived cells.* We used a trypsin/EDTA digestion method similar to that described by McAteer et al. (32). Briefly, cyst tops were excised, washed extensively in PBS, and incubated with 1× trypsin/EDTA at 37°C for 20 min. To obtain cells from individual cysts, each cyst was processed separately. In some experiments, several cysts were pooled and digested together. The tubes containing the cyst fragments were vortexed vigorously every 5 min. Thereafter, ice-cold Hanks' buffered salt solution (HBSS) containing 10% FBS was added to inactivate trypsin. The cells released from the fibrous cyst wall were washed two times with HBSS, centrifuged, resuspended in fresh culture medium, and seeded on Primaria culture plates. We also used normal human renal cortical tubule cells grown in primary culture as controls for cyst-derived epithelial cells.

To allow attachment, the cells were grown for 24–48 h in complete medium containing 10% FBS. Thereafter, the medium was changed to a defined, hormone-supplemented medium containing 2% FBS consisting of a 1:1 mixture of DMEM/Ham's F-12, supplemented with 5 μg/ml insulin, 5 μg/ml transferrin, 5 μg/ml selenium, 36 ng/ml hydrocortisone, $10^{-8}$ M triiodothyronine, 10 ng/ml epidermal growth factor, 50 ng/ml $PGE_2$, 100 U/ml penicillin, and 100 μg/ml streptomycin (50). In some experiments, the culture medium was changed overnight to a special DMEM containing 30 mM NaCl (normal medium contains 137 mM NaCl). Osmolality was maintained constant by replacing NaCl with sufficient *N*-methyl-D-glucamine. The cells were grown at 37°C in a humidified incubator in 5% $CO_2$-95% air. The culture medium was changed every 2–3 days, until confluency was reached. The cells were propagated by releasing them with 0.05% trypsin-0.53 mM EDTA and seeded on collagen I-coated culture dishes. A total of 11 polycystic kidneys were used for the present study. Because there was no difference in the characteristics of the cells cultured by these two methods, the data are combined. The cells could be propagated for three passages, after which they became senescent and stopped dividing. The majority of the experiments were performed at first or second passage on cyst-derived cells grown as primary culture. The epithelial origin of the cells was confirmed by demonstrating the presence of cytokeratin (data not shown). Two of 30 cyst cell isolates from 11 kidneys were found to be of proximal tubule origin, as evidenced by binding of FITC-labeled LTA lectin or by positive staining with an antibody against aminopeptidase N (CD13), a protein expressed by proximal tubules. The other cells were of distal tubule origin, as demonstrated by positive staining with peanut lectin (PNA) or *Dolichos biflorus* (DBA) lectin. These cyst-derived cells have also been immortalized with a chimeric adeno-SV40 virus (30). In preliminary experiments, we have shown that the immortalized ADPKD cells continue to express renin or AGT after multiple passages (30). The development and characterization of immortalized cyst-derived cells has been described (30).

*Development of rabbit anti-human renin antibody.* Anti-renin antibodies were produced against a 14-mer antigenic peptide based on the human renin sequence. Two rabbits were injected with the conjugated peptide, and antibody titers were determined 3 wk after each injection. A preimmune serum was obtained, followed by three more collections. All collected rabbit sera showed high antibody titers (1:30,000 to 1:50,000), as measured with an ELISA. The antibody was subsequently affinity purified to obtain the IgG fraction. Immunocytochemistry on renin-expressing Calu-6 cells was positive at 1:50 to 1:200 dilutions. Immunohistochemistry on formalin-fixed paraffin sections of human kidneys detected renin in afferent arterioles at 1:500 or higher dilutions. The antibody also detected renin by Western blotting in homogenates of renin-expressing cyst-derived cell lines. This antibody was used in a limited number of experiments to confirm the observations made, using a previously established antibody (6, 52).

*Immunocytochemistry of cell monolayers.* The cells were seeded on collagen I-coated coverslips and grown at 37°C for 24–48 h before study. They were washed three times with PBS and fixed for 15 min with 4% paraformaldehyde in PBS followed by three washes with PBS. The cells were permeabilized for 5 min at room temperature with 0.5% Triton X-100 in PBS, washed two times with PBS, and blocked with 0.5% BSA in PBS for 20 min. After incubation with primary antibodies for 1 h at room temperature, the cells were washed three times with PBS, followed by incubation with the FITC-labeled secondary antibodies (anti-mouse or anti-rabbit IgG, used at 1:100 to 1:200 dilution) for 30 min in the dark. The dilutions for the primary antibodies are indicated in Table 1 and in RESULTS. The cells were washed with PBS and mounted on slides, using FluoroGuard antifade mounting solution (Bio-Rad, Hercules, CA). The slides were viewed with a Zeiss Axioplan microscope equipped with epifluorescence and photographed using Kodak Elitechrome 400 film at either 8- or 15-s exposures.



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Table 2. *Primers used for PCR amplification of RAS components*

| | Name | Sequence | Position | Expected size, bp |
|---|---|---|---|---|
| AGT | Sense hAGTEX2UP | 5′-GTT CAT GCA GGC TGT GAC AG-3′ | Exon 2 | 235 |
| | Antisense hAGTEX3RP | 5′-CTC AGT GAA GGG CAC TTC AGT-3′ | Exon 3 | |
| AGT | Sense hAGTEX4UP | 5′-CAT TCT GCA CAC CGA GCT GA-3′ | Exon 4 | 258 |
| | Antisense hAGTEX5RP | 5′-ATG CTG TGC TCA GCG GGT TG-3′ | Exon 5 | |
| Renin | Sense hREN181-UP | 5′-GAG TGG AGC CAA CCC ATG AAG-3′ | Exon 2 | 217 |
| | Antisense hREN256-RP | 5′-GAG CTT GTG ATA CAC ACA GGC A-3′ | Exons 3–4 | |
| PKD1 | Sense hPKD1673UP | 5′-CCG GAT GAA GAT GAC ACC CT-3′ | Exon 37 | 254 |
| | Antisense hPKD11357RP | 5′-TCC TGC TTG ATG GCG CTT TG-3′ | Exon 38 | |
| PKD2 | Sense hPKD11186UP | 5′-CTA GCG TAT GCT CAG TTG GCA-3′ | Exon 8 | 257 |
| | Antisense hPKD11106RP | 5′-GCT GTG CCA AGT CAG ATT TCA-3′ | Exon 10 | |
| ACE | Sense hACE700UP | 5′-CTG GCG CTC CTG GTA CAA CT-3′ | Exon 5 | 251 |
| | Antisense hACE931RP | 5′-TGG TGA CAT CGA GGT TGG GC-3′ | Exon 6 | |
| $AT_1$ receptor | Sense HAT1-1714-UP | 5′-GGC TGC TCG AAG AAC AAT GTC-3′ | | 263 |
| | Antisense HAT1-3187-RP | 5′-CTA ATA GCT GAA AAC CGG CAC G-3′ | | |
| β-Actin | Sense mACTINUP105 | 5′-GTG GGC CGC TCT AGG CAC CA-3′ | Exon 1 | 242 |
| | Antisense mACTINRP344 | 5′-CGG GTT GGC CTT AGG GTT CAG-3′ | Exon 1 | |

AGT, angiotensinogen; RAS, renin-angiotensin system; PKD, polycystic kidney disease; h, human; m, mouse.

*RNA isolation and RT-PCR.* The cells were grown to confluence, washed two times in ice-cold PBS, and removed by scraping. After centrifugation, the cell pellet was snap-frozen in dry ice-ethanol, stored at −70°C, and used for RNA preparation. Total RNA was prepared, using a commercial kit (RNeasy; Qiagen, Valencia, CA). mRNA for various genes was detected by reverse transcription of the RNA to obtain the cDNA, followed by RT-PCR, using a Commercial kit. Two different pairs of oligonucleotide primers were used, corresponding to the 5′- and the 3′-regions of the genes. The primers were designed to span one or more exon-intron junctions to allow the exclusion of products amplified from genomic DNA. In all experiments, parallel tubes were used in which the RT was omitted (designated −RT); the tubes were then subjected to the same steps as those containing this enzyme (designated +RT). PCR amplifications were performed for 35 cycles, under fairly stringent conditions, using an MJ Research PTC-200 Thermocycler. Each cycle consisted of denaturation at 94°C for 30 s, annealing at 58°C for 1 min, and extension at 72°C for 1 min. In all experiments, one or more water blanks were used to verify the lack of illegitimate amplification. RT-PCR reactions were repeated with the same amount of RNA, using primers for the β-actin gene, to check for the quality of the reaction and to allow for evaluation of the amount of RNA used in each reaction. The reaction products were size fractionated on 1.5% agarose gels, stained with ethidium bromide, and photographed. The sequences of primers used are listed in Table 2.

*Gel electrophoresis and Western blots.* The cells were grown to 90% confluence on collagen-coated 100-mm culture dishes. Before experiments (15 h), the culture medium was changed to 2 ml of a serum-free medium with or without forskolin ($10^{-5}$ M) or to HBSS containing either 137 mM NaCl or to a solution with identical composition, except that it contained 30 mM NaCl. Osmolality was maintained with the addition of *N*-methyl-D-glucamine. The media were collected and the proteins concentrated after chloroform-methanol-water extraction (61). The cells were washed two times with ice-cold PBS, scraped, and collected by centrifugation. The pellet was homogenized in a small volume of lysis buffer (50 mM NaCl, 50 mM Tris·HCl, pH 8.0, 0.2% Nonidet P-40, and 0.5 mM phenylmethylsulfonyl fluoride) and incubated on ice for 60 min, followed by two freeze-thaw cycles. After centrifugation at 10,000 *g* for 10 min, the supernatant was transferred to new tubes and frozen at −70°C. In preliminary experiments, we showed that renin band intensity was much weaker in cell lysates compared with the culture medium, suggesting that renin is secreted in the medium. Only the secreted renin was studied further.

SDS-PAGE was performed according to Laemmli (26). After separation, protein bands were electrophoretically transferred to polyvinylidene difluoride membranes (Immobilon-P) according to Towbin et al. (55). Nonspecific sites were blocked by incubating the membranes for at least 2 h at room temperature in a blocking buffer consisting of Tris-buffered saline, pH 7.4, 0.2% Tween 20 (TBS-T), and 3% BSA. After two washes in TBS-T, a 1:500 to 1:1,000 dilution of the primary antibody (in blocking buffer) was added to the membranes, followed by incubation at room temperature for 2 h. After three washes in TBS-T, the second antibody was added (1:20,000 to 1:40,000 dilution of an horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG), and the membranes were incubated for 2 h at room temperature. After four washes in TBS-T, the protein bands were visualized, using the SuperSignal West Pico chemiluminescence detection method (Pierce, Rockford, IL). Membranes were briefly exposed to radiographic film for a permanent record.

*Measurement of ANG II peptide.* Cyst fluid and urine were collected in chilled containers or tubes containing, in final concentrations, 0.1% β-mercaptoethanol, 10 mM EDTA, and 10 μM 4-(2-aminoethyl)benzenesulfonyl fluoride (Sigma A845) and frozen at −20°C until assay. The samples were acidified with the addition of an equal volume of *buffer A* [1% trifluoroacetic acid (TFA), pH 4.0] and centrifuged at 12,000 *g* for 20 min at 4°C. The sample was loaded on SEP-Pack $C_{18}$ columns that had been prewashed three times with *buffer A*, and the columns were washed two times with 3 ml of *buffer A*. They were eluted with 3 ml of *buffer B* (60% acetonitrile in 1% TFA), and the eluate was evaporated overnight in a Speedvac and dissolved in RIA buffer. ANG II was measured by RIA, as previously described (5). Recovery of ANG II from the columns was determined by simultaneously passing through the columns samples that contained trace amounts of $^{125}$I-labeled ANG II (10,000 cpm/tube). In some experiments, samples were "spiked" with a known concentration (100 pg/ml) of ANG II, and processed in parallel, to allow the determination of peptide degradation.

*Reagents and supplies.* The rabbit polyclonal anti-human renin antibody (no. 74, titer 13,300) used in most of the studies was raised against purified human renin and has been described previously (6). This antibody does not cross-react with pig, dog, mouse, or rat renin (63) and has been used previously to detect renin in normal human kidney (12) and in polycystic kidneys (52). In some experiments, the findings were replicated, using a polyclonal anti-renin antibody (no. 754, titer 30,000) raised against a synthetic peptide, as described above. Rabbit polyclonal anti-human AGT antibody was a generous gift from Dr. Duane Tewksbury, Marshfield Medical Research Foun-



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Table 3. *Clinical information on subjects whose nephrectomy samples were used for study*

| Age, yr | Years on Dialysis | Duration of HTN, yr | Blood Pressure, mmHg | | Antihypertensive Medications | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Systolic | Diastolic | Diuretics | ACEIs | ARBs | β-Blockers | CCB |
| 49 | 1.5 | 12 | 132 | 80 | X | | X | | |
| 41 | 0 | 10 | 167 | 99 | X | | | X | X |
| 51 | 0.5 | 5 | 152 | 82 | X | X | | | |
| 51 | 5 | 5 | 140 | 90 | X | | X | X | |
| 51 | NK | 20 | 140 | 90 | X | | X | | |
| 62 | 10 | 15 | 150 | 90 | X | X | X | | |
| 61 | 1.5 | 41 | 116 | 49 | | | | | |
| 46 | 0 | 5 | 136 | 100 | | X | | X | |

Information could not be ascertained for 3 of the subjects. HTN, hypertension; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CCB, calcium channel blocker; NK, not known.

dation (Marshfield, WI). This antibody was not affinity purified. Mouse monoclonal antibody against ACE was purchased from Chemicon International (Temecula, CA). Mouse monoclonal antibody against ANG II type 1 receptor ($AT_1$) and mouse monoclonal antibody against ANG I and ANG II peptides were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal antibody against ANG II peptide was purchased from Peninsula Laboratories (San Carlos, CA). Human renal cortical tubule epithelial cells were purchased at *passage 1* from Clonetics (Walkersville, MD). FITC-labeled secondary antibodies (anti-mouse, anti-rabbit, or anti-goat IgG) were purchased from Pierce. FITC-labeled LTA, PNA, and DBA lectins were purchased from Sigma (St. Louis, MO). Biotin-labeled lectins and the immunohistochemistry kit (Vectastain Elite) were purchased from Vector Laboratories. RNA preparation kit (RNeasy) was purchased from Qiagen. RT-PCR kit (Access RT-PCR) was purchased from Promega (Madison, WI). Oligonucleotide primers were synthesized by GIBCO Invitrogen (Carlsbad, CA). Rat tail collagen I (catalog no. 354236) was purchased from Beckton-Dickinson (Bedford, MA). Tissue culture media and FBS were purchased from GIBCO Invitrogen. Other reagents of highest-purity grades were purchased from Sigma, Fisher, or other commercial suppliers.

*Human subjects.* Eleven polycystic kidneys were used for these experiments. The kidneys were obtained from many centers around the United States. We also used tissue specimens from two normal noncystic kidneys as controls. The nephrologists or surgeons were asked to complete a brief questionnaire on each patient, including information on hypertension and its duration, the latest available blood pressure, and antihypertensive medications. When nephrectomy was planned, the patients contacted the Polycystic Kidney Research Foundation to express interest in donating their kidney(s) for research. The investigator's laboratory was notified by the Polycystic Kidney Research Foundation, and arrangements were made for the surgical specimen to be collected and shipped to the laboratory in sterile fashion. In addition to the standard surgical informed consent, each patient signed a second informed consent to allow the use of their kidney tissue for research, including future use of archived kidney tissue. The procedures for the use of human kidneys and the consent form were approved by the Saint Louis University Institutional Review Board and was administered by the staff at the institution where the nephrectomy was performed, then signed by the principal investigator. The information on patients who provided a kidney specimen for this research is summarized in Table 3. Clinical information could be ascertained on 8 of the 11 subjects who donated a kidney. Incomplete information was provided for another patient. All patients were hypertensive at the time of nephrectomy. All except two were on maintenance hemodialysis. The main reason for nephrectomy in all patients was preparation for a kidney transplant. Despite antihypertensive treatment, five patients had systolic blood pressure ≥140 mmHg, and five patients had diastolic blood pressure ≥90 mmHg. All but one patient were treated with diuretics and antihypertensive agents before nephrectomy. Angiotensin receptor blockers were used in four patients, with two patients each receiving an ACE inhibitor and a β-blocker. Diuretics were administered in six patients.

## RESULTS

*Expression of renin by ADPKD kidneys.* In normal human kidney sections, renin staining was confined to the afferent arterioles of the glomeruli, with no staining seen in the tubules (Fig. 1). In ADPKD kidneys, we observed renin staining in cells lining the cysts and in dilated tubules. The intensity of renin staining was variable, with some cyst-lining cells showing intense staining and others showing mild staining or no staining. Only about one-half of the cysts showed renin staining (Fig. 1*A*). The available kidney sections had a few globally sclerosed glomeruli, none of which showed renin staining of the afferent arterioles. Renin staining was also not observed in afferent arterioles in areas where relatively normal glomeruli were found. By contrast, in normal human kidneys, strong renin staining was seen in the afferent arterioles of many glomeruli (Fig. 1*B*). The findings suggest that juxtaglomerular apparatus (JGA) renin may be downregulated in ADPKD kidneys. No staining was seen when sections were incubated with rabbit preimmune IgG, confirming specificity (Fig. 1*C*).

*Expression of AGT by ADPKD kidneys.* In normal human kidney tissues, AGT was seen only in proximal tubules (Fig. 2*C*). In ADPKD kidneys, we observed moderately strong AGT immunostaining in cyst-lining cells and in many proximal tubules (Fig. 2, *A* and *B*). Only some cysts, presumably of proximal origin (see below), showed AGT staining. Within a given cyst, the intensity of AGT staining was variable, with some cyst-lining cells showing only mild staining or no staining. We presume this to be a result of chronic disease, delay in tissue fixation, or antigen accessibility. The staining intensity of cysts decreased with antibody dilution, persisting down to 1:32,000 dilution, whereas proximal tubule staining persisted down to 1:64,000 dilution. Sections incubated with rabbit preimmune IgG showed no staining, confirming specificity (data not shown).

Because only a portion of cysts in each ADPKD kidney expressed renin or AGT, and because AGT is known to be expressed by proximal tubules (37), we performed additional studies to determine whether the cysts expressing renin or AGT might originate from different tubule segments. To determine the tubule origin of the cysts, adjacent sections of the same ADPKD kidney blocks were alternately stained for renin and for AGT (Fig. 3). Other adjacent sections were stained with biotinylated lectins to determine the tubule origin of the cysts



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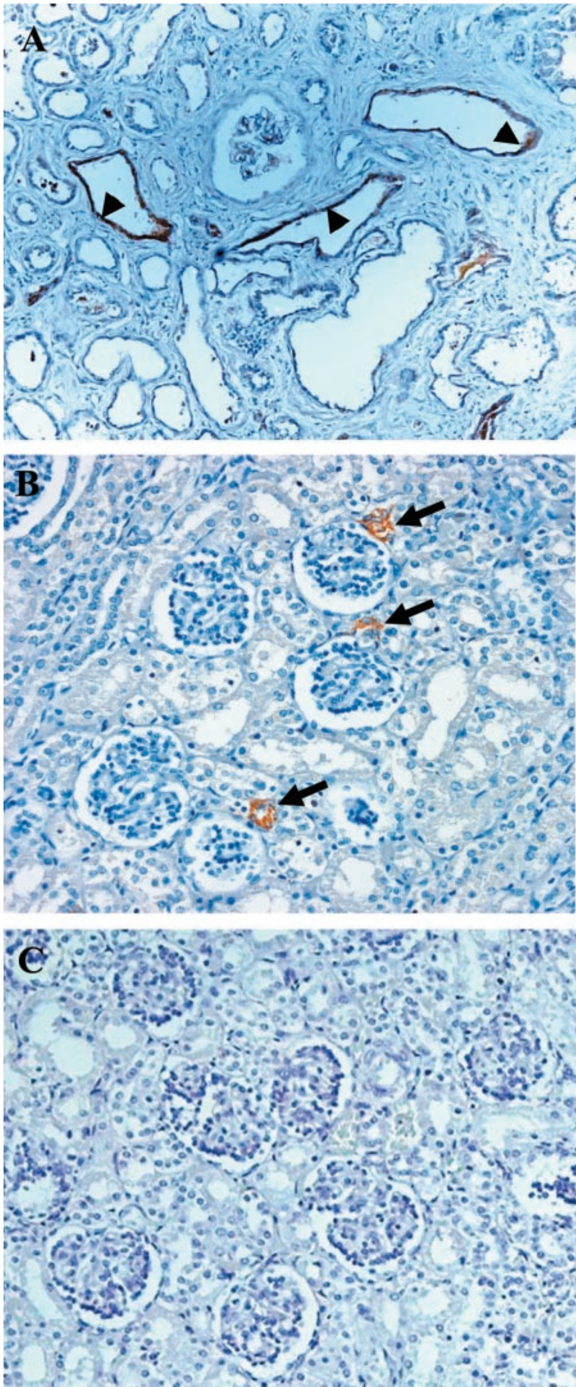


Fig. 1. Renin expression by autosomal dominant polycystic kidney disease (ADPKD) kidney. Sections from an ADPKD kidney (*A*) and a normal human kidney (*B*) stained for renin. *A*: renin staining is seen in 3 cysts (arrowheads) while several other cysts and the glomerulus at the center are not stained. Note severe interstitial fibrosis and tubular damage. *B*: in normal kidney, renin staining is confined to the afferent arterioles of glomeruli (arrows). *C*: section of normal kidney stained with preimmune IgG shows no staining. Magnification ×400.

(13, 46). We used LTA as a marker of proximal tubules and PNA as a distal tubule marker. Based on studies performed in four different ADPKD kidneys, we showed that renin and AGT are localized on different cysts. Renin was expressed by cysts of distal tubule origin, as evidenced by positive staining with PNA, whereas AGT was expressed by cysts of proximal tubule origin and by proximal tubules, as evidenced by positive staining with LTA (Fig. 3). Because a limited number of cysts were available for examination, we cannot exclude the possibility that some cysts might express both renin and AGT.

*Expression of other RAS components by ADPKD kidneys.* Using immunohistochemistry with specific antibodies, we demonstrated the presence of other components of the RAS in

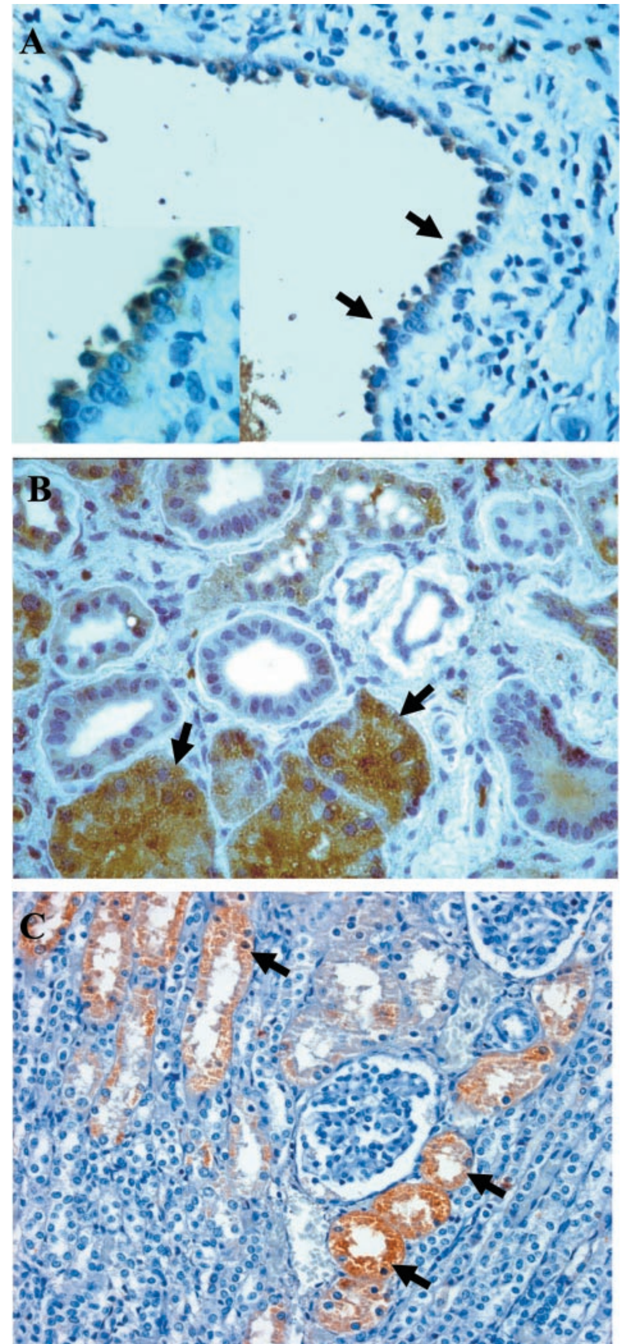


Fig. 2. Angiotensinogen (AGT) expression by ADPKD kidney. Sections from ADPKD and normal kidneys stained for AGT. *A*: portion of a cyst with AGT staining of cyst-lining cells (arrows). *Inset*: higher magnification of AGT-expressing cells. *B*: section from another ADPKD kidney showing strong AGT staining of several tubules (arrows). *C*: section from a normal human kidney showing AGT staining of proximal tubules (arrows). Magnification ×400.



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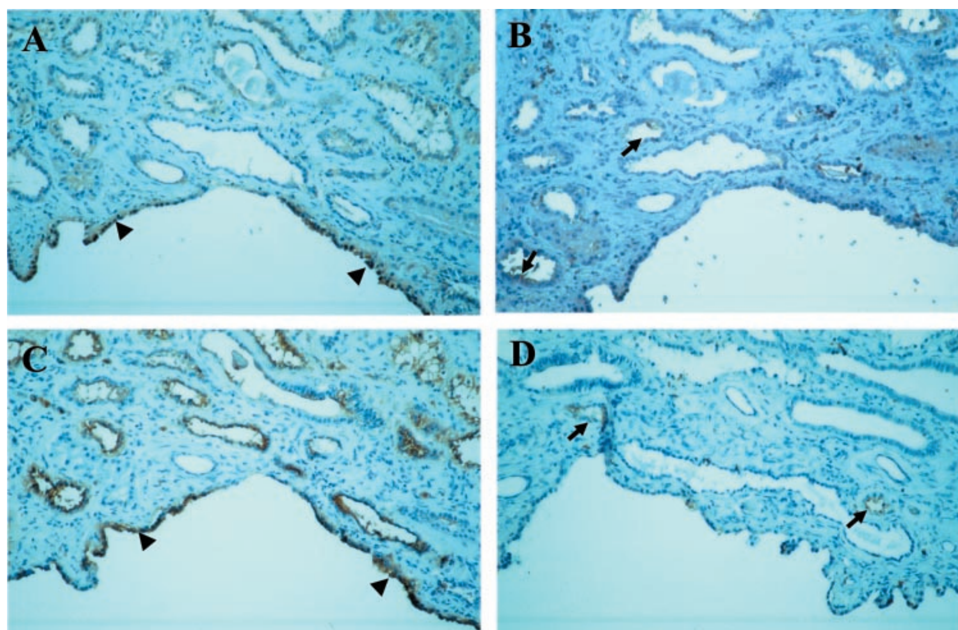


Fig. 3. Distribution of renin and AGT between cysts of ADPKD kidney. Serial sections from an ADPKD kidney stained with antibodies against renin (*A*), AGT (*B*), or with biotinylated *Arachis hypogaea* (PNA; *C*) or *Lotus tetragonolobus* (LTA; *D*) lectins. The sections show part of a large cyst and several smaller cysts and tubules. *A*: strong renin staining is seen in cells lining the large cyst (arrowheads) with weak renin staining in some of the dilated tubules. *B*: AGT staining is confined to a few tubules (arrows) with no staining of the large cyst. *C*: same area stained with PNA lectin shows strong staining of distal tubules and the large cyst (arrowheads). *D*: same area as in *C* at a slightly different depth stained with LTA lectin shows staining of two proximal tubules (arrows). Distal tubules and the large cyst are not stained. The ability of the large cyst to bind PNA lectin but not LTA lectin identifies the cyst to be of distal origin.

cyst-lining cells and in previously described locations within the kidney. We observed immunostaining for ACE in both proximal and distal tubules and in many dilated tubules and cysts (Fig. 4, *B* and *C*). In control kidney sections, ACE staining was confined to proximal tubules (Fig. 4*A*). We also observed strong immunostaining for $AT_1$ receptor in most proximal tubules and in some small- and medium-sized cysts (Fig. 4, *E* and *F*).

The presence of immunostainable AGT, renin, ACE, and $AT_1$ receptors in cysts and some dilated tubules suggests that ANG I and ANG II could be produced in situ within cysts and dilated tubules in ADPKD. To examine this possibility, we stained ADPKD kidney sections with a monoclonal antibody that recognizes both ANG I and ANG II and with a more specific polyclonal antibody against ANG II with low cross-reactivity against ANG I. Using these two antibodies, we detected ANG I and ANG II immunostaining in cyst-lining cells and within the lumen of some cysts (Fig. 5). ANG II staining was also present in tubule epithelial cells and within the lumen of proximal tubules. Some distal tubules also stained positive for ANG II (Fig. 4*C*). The results show that the components of the RAS, including the effector peptide ANG II, are present within cysts and dilated tubules of polycystic kidneys. The presence of ANG II-positive particles within the cyst lumen suggests that ANG II is either secreted in the lumen or formed within the cyst lumen from conversion of ANG I to ANG II.

If ANG II is secreted in or formed within the cyst lumen, one would expect relatively high concentrations of the peptide in cyst fluid, provided that it is not degraded. ANG II present within cysts could find its way to the lumen of the tubules to which they are connected. We therefore measured ANG II concentrations in cyst fluid collected at ~24 h after nephrectomy (Table 4). Fluids collected from 12 cysts were used for these experiments. ANG II concentration was below the detection limit of the assay in five cysts. The (mean ± SE) cyst fluid ANG II concentration in the remaining seven cysts was 1,063 ± 81 pg/ml (~1 pmol/ml). As a crude measure of ANG II in distal tubules (37), ANG II concentrations were measured in the urine of three ADPKD subjects with normal renal function but were detectable in only one subject. ANG II concentration in the urine of this individual was 88.2 ng/ml (~84 pmol/ml). In comparison, ANG II concentrations in the urine of two control subjects were 61 and 101 ng/ml (~58 and 96.6 pmol/ml).

*Cyst-derived cells in culture express renin and AGT protein.* Our findings suggest that, in ADPKD, the components of the RAS can be produced by cysts and dilated tubules, but we cannot rule out nonspecific uptake by cyst-lining cells. Plasma renin (mol wt ~30,000) is partially filtered at the glomerulus and degraded in the proximal tubule (25). The presence of renin within the cyst epithelium could therefore represent endocytic uptake of filtered renin from the lumen. AGT (mol wt ~60,000) is not filtered but can be produced by proximal tubules. Therefore, it might reach the microcysts attached to the tubules in a retrograde fashion and endocytosed by cyst-lining cells. To prove that cyst-lining cells synthesize renin and AGT, we isolated epithelial cells from multiple individual cysts of 11 polycystic kidneys. Both ADPKD cells in primary culture and immortalized ADPKD cells were used for the studies described below.

Using immunohistochemical techniques, we showed strong renin immunostaining in the majority of cyst-derived cells in culture. Diffuse renin staining was observed within the cytoplasm of the cells. When the tubule origin of the cells was identified, renin appeared to be expressed in cells derived from distal cysts (Fig. 6*A*). No renin staining was seen in cells from proximal cysts (Fig. 6*B*). Calu-6 cells, a lung carcinoma cell line known to express renin (28), showed strong renin staining (Fig. 6*C*). No staining was seen when preimmune rabbit IgG was used at the same dilution (data not shown). Renin continued to be expressed in at least 11 cell isolates after they had been immortalized and passaged multiple times.



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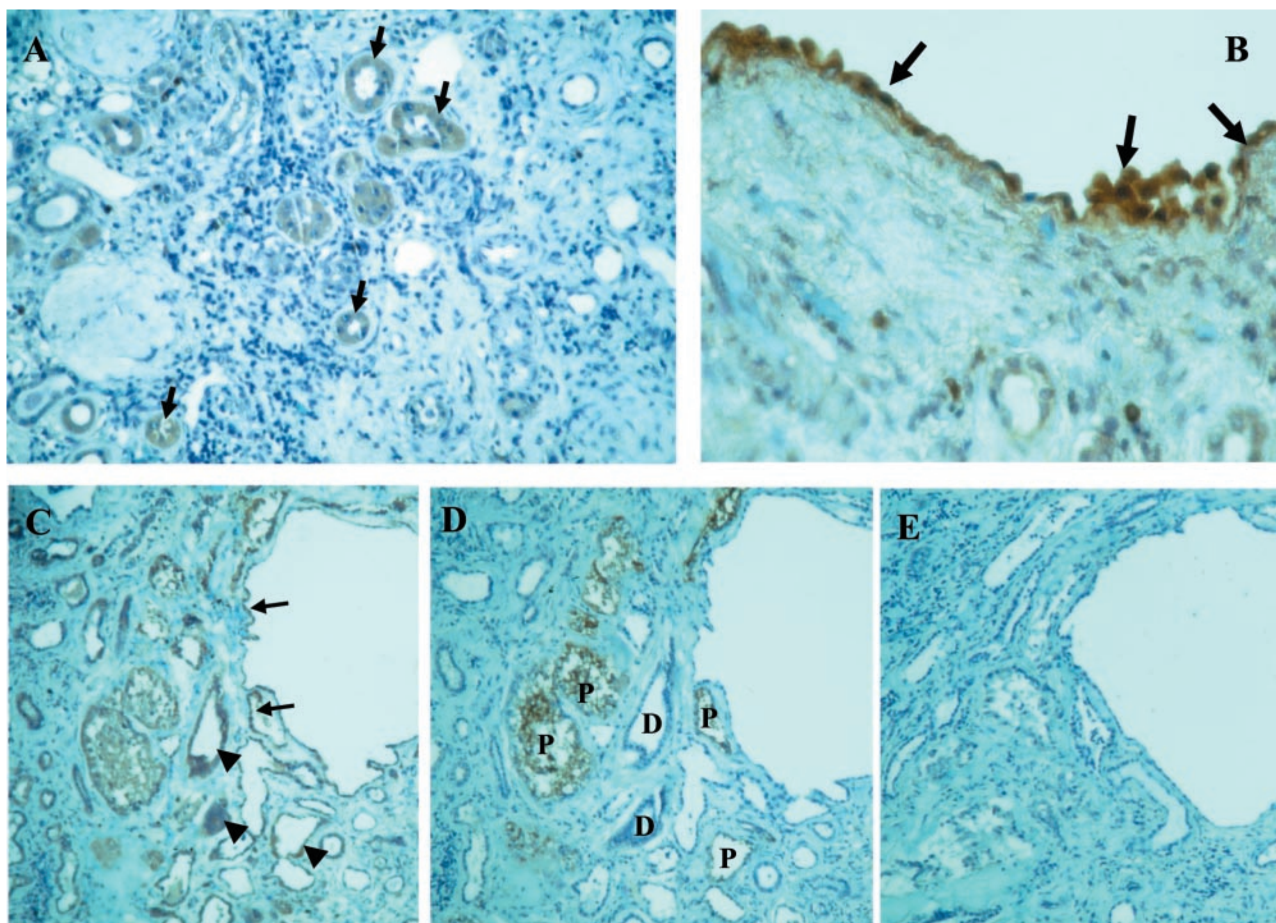


Fig. 4. ANG II expression by ADPKD kidney. *A*: normal control kidney showing mild to moderate ANG II expression in several tubules (arrows). *B*: section from an ADPKD kidney containing part of a large cyst, displaying strong ANG II staining of cyst-lining cells (arrows). *C–E*: serial sections of the same region of an ADPKD kidney stained with antibodies against ANG I and ANG II (*C*), biotinylated LTA lectin (*D*), or preimmune IgG (*E*). ANG II staining is seen in portions of a cyst (arrows) and in dilated tubules (arrowheads) identified as both proximal (LTA+) and distal (LTA−) tubules based on lectin-binding characteristics (compare *C* with *D*). No staining is obtained when preimmune IgG is used (*E*). P, proximal; D, distal. Magnification ×400.

We further observed moderately strong AGT immunostaining in four cell isolates (9–7, 8–3, 18–5, and 19–9). Similar to renin, AGT staining showed a diffuse and variable cytoplasmic pattern (Fig. 6). AGT was expressed in cells derived from proximal cysts (Fig. 6*E*). There was no AGT staining or only mild staining in cells derived from distal cysts (Fig. 6*D*). The control human renal cortical epithelial cell line showed variable AGT staining (Fig. 6*F*), suggesting that it is composed of both proximal and distal tubule epithelial cells. AGT continued to be expressed in the same cells after they had been immortalized and passaged multiple times. These in vitro cell culture results confirm renin and AGT synthesis by cyst-lining cells.

*Cyst-derived cells express ACE, ANG II, and $AT_1$ protein.* To determine whether other RAS components are expressed by cyst-derived cells in culture, we used specific antibodies raised against each of these components in a two-step immunocytochemical method (Fig. 7). Staining for ACE was primarily localized to the periphery of the cells (Fig. 7*A*). ANG II and $AT_1$ staining was seen in all the cells examined, showing a diffuse cytoplasmic pattern (Fig. 7, *B* and *C*). Similar results were obtained in immortalized cyst-derived cells (data not shown). These immunocytochemistry results, therefore, corroborate our findings in ADPKD kidney tissue sections. We conclude that cyst-lining cells are capable of synthesizing the components of the RAS, including ANG I and ANG II.

*Demonstration of renin in cyst-derived cells by Western blot analysis.* We further explored renin production by renin-expressing cyst-derived cells using Western blot analysis (Fig. 8). Calu-6 cells, a lung carcinoma cell line known to express renin, were used as the control (28). The cells were grown in the presence of media containing either 137 mM NaCl (normal Na) or 30 mM NaCl (low Na). Some cells were grown in the presence or absence of forskolin ($10^{-5}$ M). Culture media were collected and used for SDS-PAGE, followed by Western blotting. Renin was seen as a band of ~30,000 Da. Renin band intensity was higher in media from cells exposed to a low NaCl concentration compared with cells kept in a normal NaCl concentration. In Calu-6 cells, low sodium resulted in either no change or a decline in renin band intensity. Similar results were obtained in two experiments. The results suggest that renin is produced by cyst-lining cells and could be secreted in the cyst



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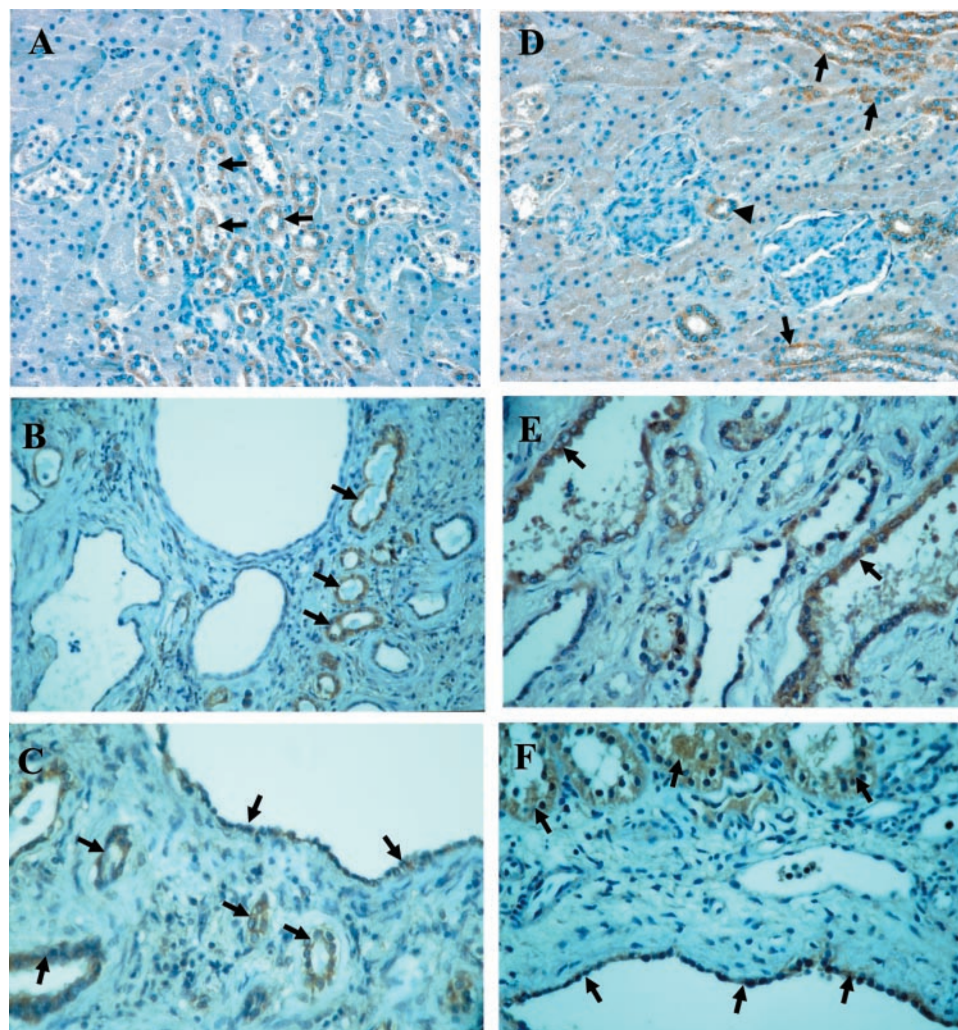


Fig. 5. Expression of angiotensin-converting enzyme (ACE; *A–C*) and ANG II type 1 receptor ($AT_1$; *D–F*) by ADPKD kidney. *A*: section from a normal kidney showing mild to moderate ACE staining of several tubules (arrows). *B*: section from an ADPKD kidney showing ACE staining in several dilated tubules (arrows). The two cysts at the center are not stained. *C*: section from another ADPKD kidney showing ACE staining of both tubules and a large cyst (arrows). *D*: section from a normal kidney showing staining for $AT_1$ receptors in tubules (arrows) and in afferent arteriole (arrowhead). *E*: section from an ADPKD kidney showing strong $AT_1$ staining in cystic dilated tubules (arrows). *F*: ADPKD kidney showing $AT_1$ staining of a cyst in addition to several tubules (arrows). Magnification ×400.

lumen. Furthermore, renin production by cyst-derived cells could be regulated by extracellular NaCl concentrations.

*Cyst-derived cells express renin and AGT mRNA.* If renin and AGT are synthesized by the cyst epithelium, one would expect the presence of mRNA for these proteins in cyst epithelium and in cyst-derived cells in culture. The presence of renin and AGT mRNA was evaluated by RT-PCR of total RNA isolated from cultured cyst-derived cells. Renin mRNA was expressed by the majority of cell isolates studied (Figs. 9 and 10*A*). Furthermore, renin mRNA expression appeared to be higher in cells grown on collagen I-coated plates compared with cells grown on plastic (Fig. 9*A*). AGT mRNA was expressed by only a few cyst-derived cell isolates (Fig. 9*B*).

In addition to mRNA for renin and AGT, RT-PCR analysis of RNA isolated from cultured cyst-derived cells showed the presence of mRNA for ACE and for $AT_1$ in all cyst-derived cells studied (Fig. 9, *C* and *D*), regardless of tubule origin.

*Differential expression of renin and AGT by cysts.* We found that, in general, renin-expressing cells did not express AGT and AGT-expressing cells did not express renin. Using tubule-specific lectins, we confirmed that AGT mRNA is expressed by cyst-derived cells of proximal tubule origin, whereas renin mRNA is expressed by cyst-derived cells of distal tubule origin (Fig. 10*A*). For those cells in which both immunocytochemistry and RT-PCR data were available, there was concordance between renin and AGT protein and mRNA expression. For example, cyst-derived cells of distal tubule origin (e.g., 11–6) express renin mRNA at the exclusion of AGT mRNA (Fig. 10*A*). The same cells also show moderately strong renin staining by immunocytochemistry but no staining for AGT (Fig. 10*B*).

Table 4. *ANG II concentrations in cyst fluid and urine*

| Cyst Fluid | ANG II Concentration, pg/ml | Urine | ANG II Concentration, ng/ml |
|---|---|---|---|
| *Cyst 6* | BDL | U1* | BDL |
| *Cyst 7* | 1,415 | U2* | BDL |
| *Cyst 8* | 1,223 | U3* | 88.2 |
| *Cyst 9* | BDL | | |
| *Cyst 10* | 739 | | |
| *Cyst 11* | BDL | | |
| *Cyst 12* | BDL | U1† | 61.1 |
| *Cyst 13* | BDL | U2† | 101.3 |
| *Cyst 14* | 1,022 | | |
| *Cyst 15* | 1,076 | | |
| *Cyst 16* | 1,033 | | |
| *Cyst 17* | 936 | | |

BDL, below detection limit of the assay. Urine values are not corrected for Cr concentration. *ADPKD urine. †Control urine.



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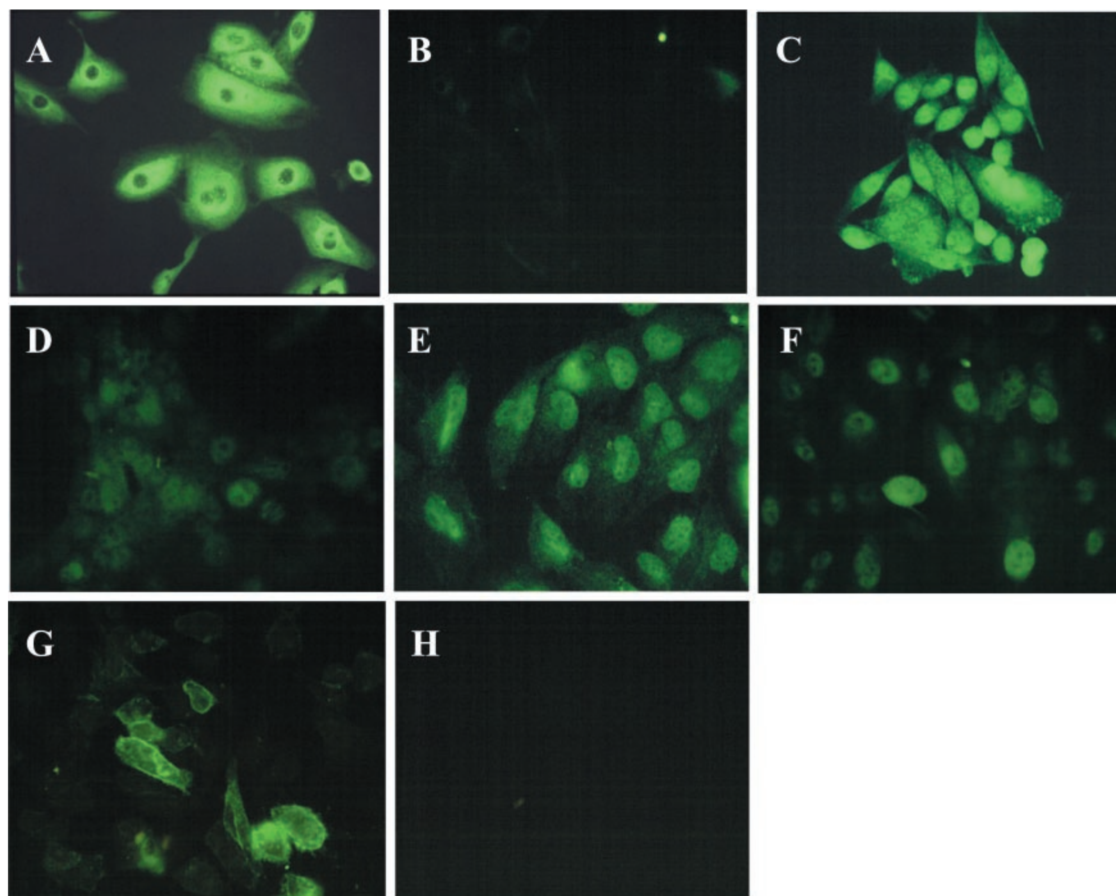


Fig. 6. Expression of renin and AGT by cyst-derived cells in culture. *A*: cells derived from a distal cyst (9–9) show strong renin staining. *B*: cells from a proximal cyst (9–7) do not express renin. *C*: Calu-6 cells show strong renin staining. *D*: cells from a distal cyst (9–9) show weak or no AGT staining. *E*: cells from a proximal cyst (19–9) show strong AGT staining. *F*: control human cortical epithelial cell line (RCTEC) shows AGT staining in about one-half of the cells, presumably of proximal tubule origin. *G*: cyst-derived cells (9–7) shown in *B* express CD13, identifying them to be of proximal origin. *H*: proximal cyst cells (9–7) stained with preimmune IgG. Magnification ×400.

Taken together, the results indicate that, in ADPKD, cyst-lining epithelia can synthesize the components of the RAS. This is associated with production and secretion of ANG II by cysts and a relatively high intratubular ANG II concentration.

## DISCUSSION

The mechanism of hypertension in ADPKD remains poorly understood. Involvement of the RAS has been postulated, but the data correlating plasma renin activity with blood pressure in ADPKD patients are inconclusive. Although some studies show increased plasma renin activity in hypertensive ADPKD patients compared with control subjects, a strong association has not been established in all studies (7, 22), casting doubt on the role of systemic RAS in blood pressure regulation in ADPKD.

*The role of intrarenal RAS in hypertensive disorders.* It is now well accepted that increased activity of the intrarenal

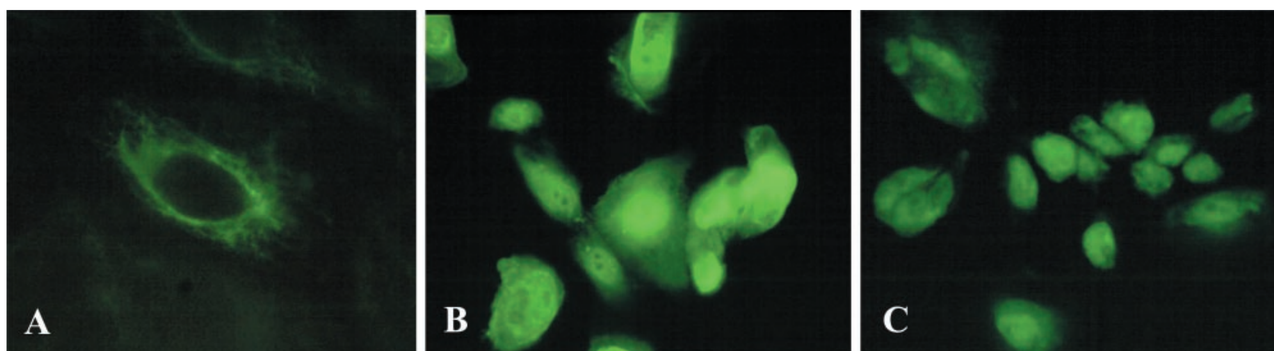


Fig. 7. Expression of other renin-angiotensin system (RAS) components by cyst-derived cells in culture. *A*: cells derived from a distal cyst (9–9) show moderately strong staining for ACE, taking a peripheral membrane-associated pattern. *B*: cells from a proximal cyst (19–9) show strong ANG II staining. *C*: same cells (19–9) also express $AT_1$ receptors. Magnification ×400.



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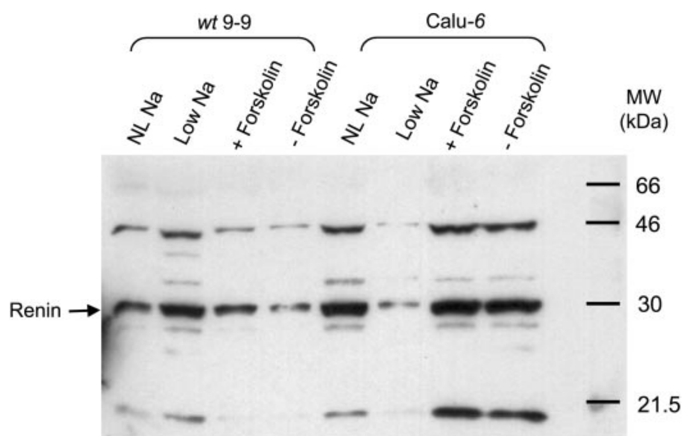


Fig. 8. Demonstration of renin in cyst-derived cells in culture by Western blotting. Renin-expressing immortalized cyst-derived cells (wt 9–9) were grown in the presence of culture media containing 137 mM NaCl [normal Na (NL Na)] until confluent and then either kept in normal-sodium medium or switched to a medium containing 30 mM NaCl (low Na). The cells were also grown in the presence or absence of forskolin ($10^{-5}$ M). Calu-6 cells, a lung carcinoma cell line known to express renin, were used as control. Culture media were collected, concentrated, and used for SDS-PAGE, followed by Western blotting. Protein (30 μg) was loaded in each lane. Renin is seen as a band of ~30,000. Renin band intensity is increased in ADPKD cells exposed to low NaCl concentrations compared with cells exposed to normal NaCl concentrations. Renin band intensity is somewhat increased in cells exposed to forskolin to increase intracellular cAMP. *Right*: renin expression in Calu-6 cells does not change with forskolin. Lower renin band intensity in low-sodium medium is likely because of uneven protein loading.

rather than the systemic RAS is responsible for many forms of hypertension (11, 37, 58). It has been suggested that persistent elevation of intrarenal ANG II production with an inability to reduce ANG II formation in response to a high sodium intake will result in resetting of the pressure-natriuresis relationship toward higher blood pressures, thus leading to hypertension (18, 20). Several lines of evidence suggest that a similar paradigm may apply to polycystic kidney disease. In ADPKD patients, the pressure-natriuresis curve is shifted to the right along with increased blood pressure sensitivity to salt loading (44, 54). Sodium retention and volume expansion have been reported in hypertensive ADPKD patients, before the onset of renal failure (35, 55). These patients also have higher renal vascular resistance compared with normotensive controls and an exaggerated vascular response to converting-enzyme inhibition (7, 53, 60). Additionally, in normotensive ADPKD patients, the renal vasculature is insensitive to ANG II infusion, with a lower relative rise in vascular resistance compared with control subjects (2). Taken together, these observations suggest increased intrarenal ANG II production in ADPKD. Because the majority of tubules continue to function, high ANG II concentrations within the tubule lumen could result in increased renal tubular sodium reabsorption and hypertension if salt intake is not reduced (19, 20).

*Overexpression of RAS in polycystic kidneys.* In the present study, we show that, in ADPKD, the components of the RAS can be produced by cysts and dilated tubules. Renin expression is confined to distal cysts and some dilated distal tubules, whereas AGT expression is confined to proximal cysts and proximal tubules. Because AGT is known to be present in proximal tubules, its expression by cysts of proximal tubule origin is not surprising. Similarly, renin expression by cysts of distal tubule origin is in line with recent reports of renin expression in mouse connecting and collecting tubules (43). We have recently made similar observations in kidneys of *pcy* mice, considered a model of polycystic kidney disease (unpublished observations).

Several lines of evidence suggest that renin, AGT, and other RAS proteins are synthesized by cyst epithelium in ADPKD kidneys: *1*) the cultured cells used for the present studies were derived from large superficial cysts that are cut off from the

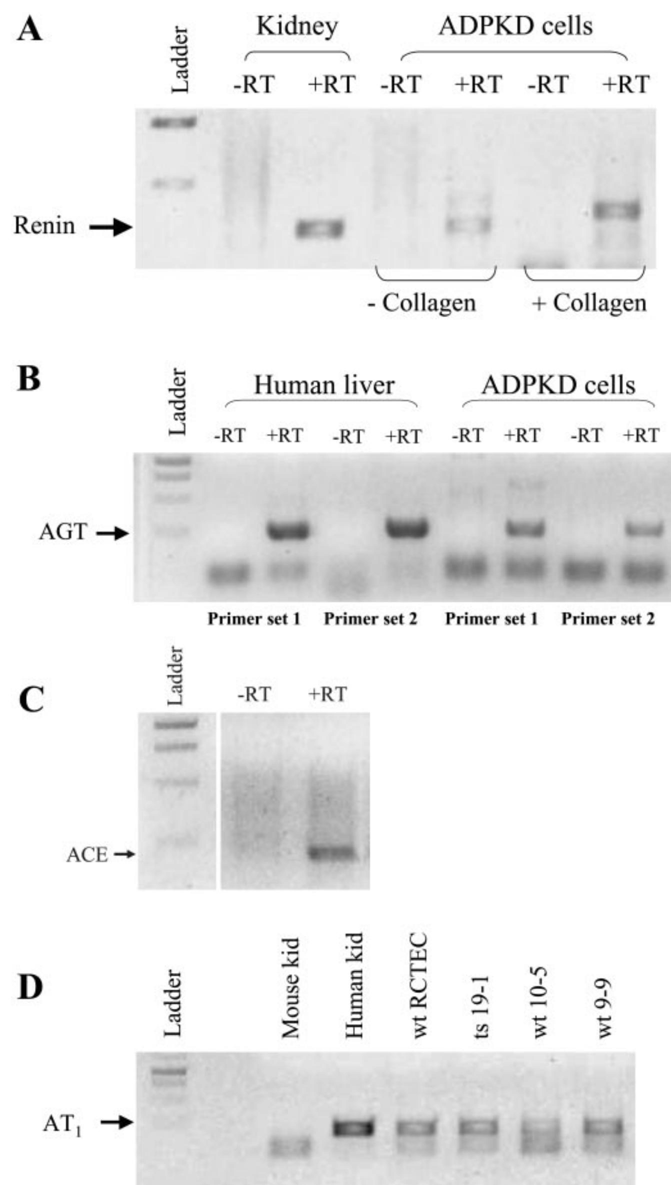


Fig. 9. Expression of renin, AGT, and ANG II receptor mRNA by cyst-derived cells in culture. Cells were isolated from individual cysts and used either as primary culture or after they had been immortalized. RT-PCR was performed with specific oligonucleotide primers for renin, AGT, ACE, or $AT_1$ receptor. *A*: renin mRNA band shows a higher intensity in ADPKD cells grown on collagen compared with cells grown on plastic. Human kidney mRNA is used as a positive control for renin. *B*: AGT mRNA band is seen in cyst-derived cells, using two different primer sets. Human liver mRNA is used as positive control for AGT. *C*: ACE mRNA is seen in a cyst-derived cell. *D*: $AT_1$ mRNA band is seen in 3 different immortalized cyst-derived cells and in the control human renal cortical epithelial cell line (wtRCTEC). −RT, control RNA sample, not exposed to RT; +RT, samples treated with RT before PCR.



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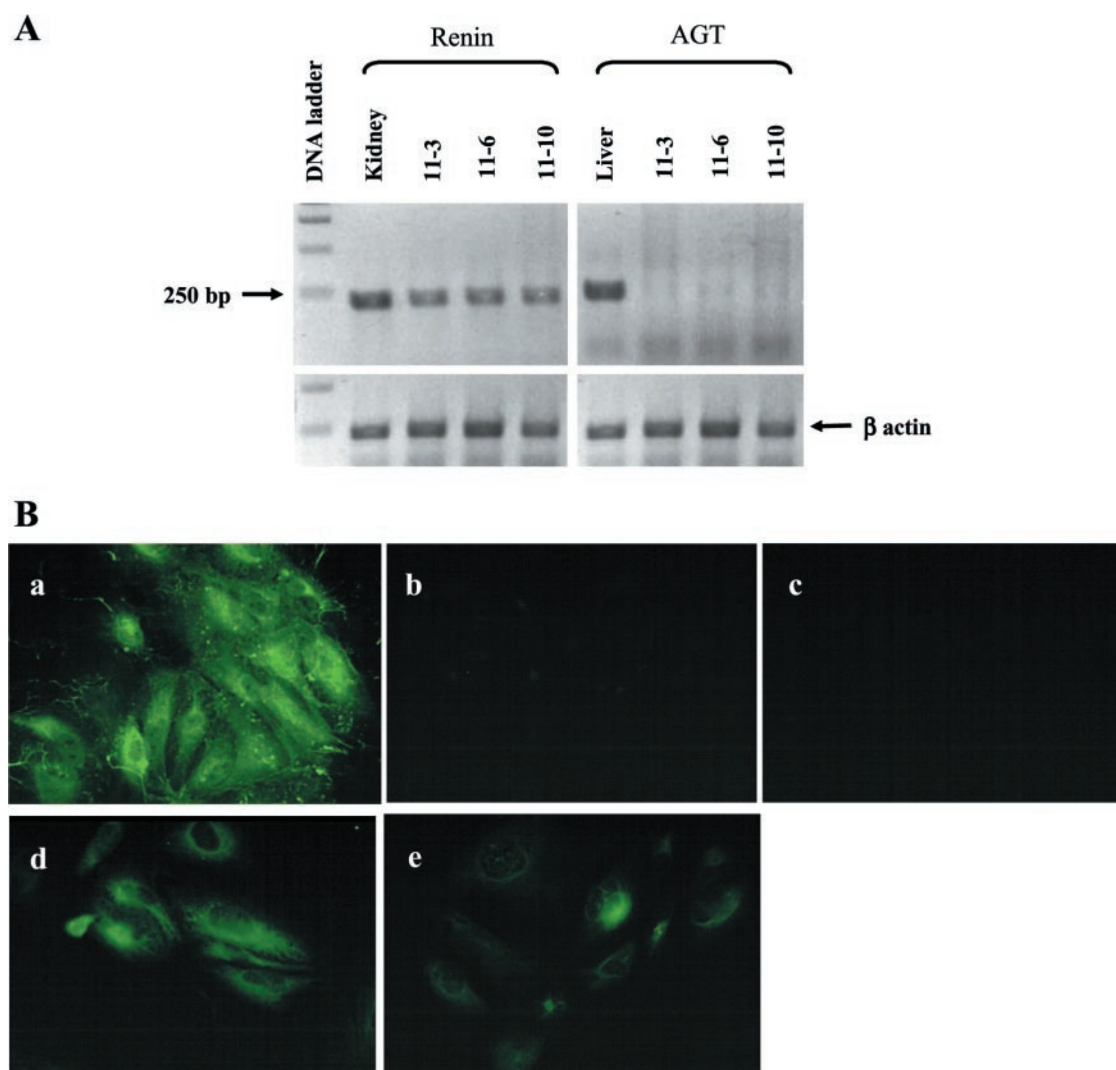


Fig. 10. Differential expression of renin and AGT mRNA in cyst-derived cells. *A*: mRNA analysis. Three immortalized cyst-derived cells, previously characterized to be of distal tubule origin (30), were used for RT-PCR analysis, using oligonucleotide primers for renin and AGT. Renin mRNA is expressed in all 3 cyst-derived cells and in human kidney, used as positive control. By contrast, none of the 3 cyst-derived cells of distal origin express AGT mRNA. A strong AGT mRNA band is seen in human liver. *B*: immunohistochemistry of renin and AGT in one of the cyst-derived cells (11–6) of distal tubule origin shown in *A*. The distal origin of the cells is confirmed by positive staining with PNA lectin (*a*) and negative staining with LTA lectin (*b*). The cells show moderately strong staining for renin (*d*) but only sparse staining for AGT (*e*). No staining is seen when preimmune IgG is used as primary antibody (*c*).

rest of the nephron, eliminating the possibility of renin absorption from the tubule lumen; *2*) renin and AGT were still detected after the cells were grown in primary culture for at least 1 wk; and *3*) immortalized cyst-derived cells continued to express RAS components after multiple passages (30).

Torres et al. (52) described ectopic production of renin by tubulocystic epithelium in ADPKD. Renin appeared to be secreted in the cyst fluid, with a higher concentration in gradient cysts (52). There was a negative correlation between cyst fluid sodium concentration and cyst fluid active renin (52). This observation suggests that cyst renin production may be regulated by changes in extracellular sodium concentration. Our Western blot analysis provides evidence for regulated release of renin by the cyst epithelium. Renin secretion was increased in cyst-derived cells exposed to low NaCl concentrations. Although Torres et al. (52) reported renin expression in both proximal tubules and in cysts of distal tubule origin, our results suggest that renin is primarily, if not exclusively, produced by cysts of distal tubule origin. Rohrwasser et al. (43) showed low levels of immunoreactive renin in mouse connecting and cortical collecting tubules, which could be augmented acutely by dietary sodium restriction. The finding that dilated distal tubules and cysts of distal tubule origin both produce renin is in agreement with the above observation and may represent derepression of renin release by distal tubules after cystic transformation.

Graham and Lindop (17) have reported increased numbers of renin-secreting cells in ADPKD kidneys with immunoreactive renin extending beyond the afferent arterioles and in small renal arteries. Contrary to these earlier reports, we seldom observed prominent JGA or arteriolar renin staining in our ADPKD kidney specimens, even though several patients were



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treated with diuretics, angiotensin receptor blockers, or ACE inhibitors (Table 3). A possible explanation is that many glomeruli were scarred or destroyed in these end-stage kidneys, leading to involution of their arterioles. However, even in kidney sections where intact glomeruli were available, JGA renin was reduced or absent (Fig. 1*A*). Increased renin expression by renal arterioles is also difficult to reconcile with the observations that systemic renin activity is not generally increased in ADPKD patients.

*Paracrine intrarenal RAS in ADPKD.* In the early stages of ADPKD, only a minority of tubules undergo a "second hit" somatic mutation and develop into cysts over several decades (40). The majority of nephrons remain normal and presumably functional before becoming damaged by compression from adjacent growing cysts. Because a significant number of cysts are connected to tubules (49), AGT produced by cystic and noncystic proximal tubules could gain access to distal tubules (43). Therefore, a paracrine system could exist whereby AGT produced by proximal tubules and proximal cysts could reach the distal tubules where it is cleaved by renin from distal cysts to form ANG I (see Fig. 11). ACE is present on the brush border of proximal tubules and within distal tubules (4), allowing ANG I to be cleaved readily to ANG II. ACE-independent, chymase-mediated ANG II formation has also been reported in the interstitium of ADPKD kidney tissues (33). ANG II could bind to apical $AT_1$ receptors at these tubule sites and increase sodium and water reabsorption (15, 24, 51, 57). Although 24-h delay in sample collection may have resulted in significant degradation of ANG II, relatively high ANG II concentrations were measured in fluids collected from 7 of the 12 cysts studied, but ANG II remained undetectable in 5 cysts (Table 4). For those cysts in which ANG II could be measured, the average ANG II concentration in cyst fluid was 1 pmol/ml, well within the range of tubular ANG II reported previously (36, 38). These concentrations are also higher than those measured in plasma (38), suggesting in situ production by cyst-lining epithelium. If one considers that a significant fraction of tubular ANG II is derived from cysts draining in the tubules, the ANG II concentrations measured here are well within the range that can stimulate tubular sodium reabsorption.

Previous studies in cyst epithelium have demonstrated the presence of apical chloride channels and reversed polarity of $Na^+$-$K^+$-ATPase pumps with an apical instead of basolateral location (62). The net effect of these channels is sodium and chloride secretion in the cyst lumen and cyst growth (39, 62). The mechanism described here might appear incompatible with overall sodium and chloride reabsorption by the renal tubules. Because $<1\%$ of tubules develop into cysts (40), the contribution of the secretory flux described above is negligible compared with the ANG II-stimulated absorptive flux. Therefore, the net effect of increased intrarenal RAS activity would be sodium and water retention. Recently, it was shown that ADPKD cyst-lining epithelial cells absorb sodium by an amiloride-sensitive pathway (42). Because of similarity of ADPKD and ADPKD, it is likely that cysts in ADPKD can also absorb sodium.

*Proposed mechanism of hypertension in ADPKD.* Based on the findings of this study and the classical work of Guyton and Hall (19, 20), we propose the following mechanism for the development of hypertension in ADPKD (Fig. 11). Ectopic and excessive production of AGT and renin by proximal and distal cysts could lead to increased intratubular ANG II concentrations and excessive renal tubular sodium reabsorption (37). Renin production may not become suppressed when dietary sodium is increased. This could lead to a shift of the pressure-natriuresis curve to the right and chronic hypertension, unless the dietary sodium intake is reduced. Increased ANG II production may also contribute to cellular proliferation and cyst growth, and to interstitial fibrosis (39). The mechanism described above might contribute to hypertension in early stages of ADPKD. Later in the course of the disease, hypertension could be sustained by a combination of factors that include salt and water retention and RAS overactivity resulting from nephron damage.

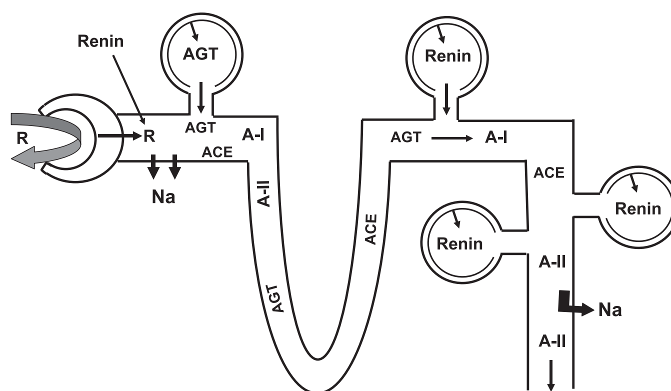


Fig. 11. Speculative model of autocrine/paracrine tubular RAS in polycystic kidney disease. AGT is produced by proximal tubules, where it can be cleaved to ANG I by filtered renin (R). ANG I is converted to ANG II by ACE present on the brush-border membrane. ANG II binds to apical $AT_1$ receptors to stimulate sodium reabsorption via apical $Na^+/H^+$ antiporter. In early stages of disease, many microcysts maintain continuity with the rest of the nephron. Accordingly, AGT produced by proximal tubules and proximal cysts can reach the distal tubules where it can be cleaved by renin emerging from distal cysts and dilated distal tubules. Cleavage of AGT by renin results in the formation of ANG I, which is readily converted to ANG II by ACE, present in this nephron segment. ANG II produced at distal tubule sites can lead to increased sodium reabsorption via stimulation of the epithelial sodium channels. Excessive sodium intake may influence ANG II production/accumulation by *1*) decreasing AGT production by proximal tubules and cysts and *2*) reducing renin production by distal tubules and cysts. Inability of polycystic kidneys to downregulate the proximal and distal limbs of this paracrine intratubular RAS could result in sustained increases in intratubular ANG II levels. One can speculate that increased ANG II could lead to salt and water retention and hypertension.

*Possible mechanisms of intrarenal RAS upregulation in ADPKD.* Changes in extracellular NaCl or intracellular calcium have been shown to regulate renin production in juxtaglomerular cells (43). Polycystin-2 may represent a component of a nonselective sodium or calcium channel (16, 34), which requires assembly with polycystin-1 for adequate ion channel function (21). Therefore, it is possible that the polycystins could be involved in the regulation of renin production by modulating sodium or calcium fluxes across the cyst epithelium. Defective channel activity resulting from specific polycystin mutations (9) could reduce intracellular sodium and calcium concentrations and stimulate renin production. In JGA cells, renin release is also controlled by changes in early distal tubular flow (29). Recently, Nauli et al. (36) showed that polycystins act as flow sensors in renal tubules, transducing mechanical fluid flow signals into calcium signals. In cultured



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cells with a homozygous polycystin mutation, the cilia fail to sense fluid flow (36). We also speculate that increased renin expression by cyst-lining epithelia might be related to failure of mechanosensation resulting from polycystin mutations.

The mechanism of ectopic AGT expression in ADPKD remains speculative. In cardiac myocytes, mechanical stretch activates the AGT gene expression and the formation of ANG II, which is involved in stretch-induced myocardial hypertrophy (48). Hypoxia can also stimulate AGT gene expression (27). It is possible that mechanical stretch or hypoxia could exert similar effects on cyst-lining cells. The polycystin complex triggers the formation of a number of transcription factors. One could speculate that polycystic kidney disease mutations may also interfere with the formation of *trans*-acting factors that can regulate AGT gene expression. Recent isolation of immortalized cyst-derived cells from ADPKD kidneys (30) should provide the tools necessary to test these hypotheses.

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