Original Article

Urinary Renin in Patients and Mice With Diabetic Kidney Disease

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Abstract—In patients with diabetic kidney disease (DKD), plasma renin activity is usually decreased, but there is limited information on urinary renin and its origin. Urinary renin was evaluated in samples from patients with longstanding type I diabetes mellitus and mice with streptozotocin-induced diabetes mellitus. Renin-reporter mouse model (Ren1d-Cre;mT/mG) was made diabetic with streptozotocin to examine whether the distribution of cells of the renin lineage was altered in a chronic diabetic environment. Active renin was increased in urine samples from patients with DKD (n=36), compared with those without DKD (n=38; 3.2 versus 1.3 pg/mg creatinine; $P<0.001$). In mice with streptozotocin-induced diabetes mellitus, urine renin was also increased compared with nondiabetic controls. By immunohistochemistry, in mice with streptozotocin-induced diabetes mellitus, juxtaglomerular apparatus and proximal tubular renin staining were reduced, whereas collecting tubule staining, by contrast, was increased. To examine the role of filtration and tubular reabsorption on urinary renin, mice were either infused with either mouse or human recombinant renin and lysine (a blocker of proximal tubular protein reabsorption). Infusion of either form of renin together with lysine markedly increased urinary renin such that it was no longer different between nondiabetic and diabetic mice. Megalin mRNA was reduced in the kidney cortex of streptozotocin-treated mice (0.70±0.09 versus 1.01±0.04 in controls, $P=0.01$) consistent with impaired tubular reabsorption. In Ren1d-Cre;mT/mG with streptozotocin-induced diabetes mellitus, the distribution of renin lineage cells within the kidney was similar to nondiabetic renin-reporter mice. No evidence for migration of cells of renin linage to the collecting duct in diabetic mice could be found. Renin mRNA in microdissected collecting ducts from streptozotocin-treated mice, moreover, was not significantly different than in controls, whereas in kidney cortex, largely reflecting juxtaglomerular apparatus renin, it was significantly reduced. In conclusion, in urine from patients with type 1 diabetes mellitus and DKD and from mice with streptozotocin-induced diabetes mellitus, renin is elevated. This cannot be attributed to production from cells of the renin lineage migrating to the collecting duct in a chronic hyperglycemic environment. Rather, the elevated levels of urinary renin found in DKD are best attributed to altered glomerular filteration and impaired proximal tubular reabsorption.
(***Hypertension***. **2019;74:00-00. DOI: 10.1161/HYPERTENSIONAHA.119.12873.)** • **Online Data Supplement**

Key Words: diabetes mellitus ■ kidney ■ mice ■ renin ■ renin-angiotensin system

Renin is the key physiologically regulated enzyme of the cascade that culminates in activation of the renin-Ang (angiotensin) system (RAS).[1–14] Under normal physiological conditions, juxtaglomerular cells are the main, if not the only source of circulating, active renin, constituting, therefore, the endocrine branch of RAS.[4,6,8] On reaching the circulation, renin generates Ang I by hydrolyzing its unique substrate angiotensinogen, which is an indispensable step for the subsequent formation of Ang II.[7,15,16] Thus, renin release by secretory cells in the juxtaglomerular apparatus (JGA) is the rate-limiting step in the activation of systemic RAS. Most RAS components are present at the tissue level, and this system moreover is believed to be overactive in kidneys from rodent models and patients with diabetic kidney disease (DKD).[17–26] By contrast, plasma renin activity (PRA) is usually low in patients with diabetic complications[27–30] This is known as the renin-paradox.[27,31,32] Despite the low/normal levels of circulating PRA, RAS blockers are effective in slowing down the progression of kidney disease.[26,33–35] This beneficial effect reflects their direct suppressive actions of the kidney RAS which is in addition to their systemic effects.

The presence of renin was described in proximal and collecting tubules as early as 1982.[36] More recently, there has been an interest in urinary renin and its possible source, which could at least, in part, be of tubular origin.[19,37–39] There is limited information on urinary renin in patients with diabetes mellitus and renal complications. It is possible that urinary renin, if increased, could contribute to activation of the RAS system,

Received February 19, 2019; first decision March 5, 2019; revision accepted April 2, 2019.

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The online-only Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/HYPERTENSIONAHA.119.12873.

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a feature of diabetic and nondiabetic CKD. By cleaving angiotensinogen that is either filtered, locally formed, or both, renin could activate RAS locally within the kidney, which is relevant to sodium retention, hypertension, glomerular hemodynamics, inflammation and progression of CKD. We, therefore, reasoned that the source of urinary renin in DKD deserves further investigation. We hypothesized that urinary renin in DKD is either the result of increased filtration because of alterations to the glomerular permeability or impaired proximal, tubular reabsorption, or both. We also wanted to examine the possibility that urinary renin could be of tubular origin, that is locally produced by tubular segments, namely in the collecting ducts (CD). In this regard, it is important to note that the PRR (prorenin receptor), a dual receptor for renin and prorenin, is present in CD cells.[19,40–43] Genetic ablation of the PRR in CD cells attenuates the hypertensive response to Ang II infusions suggesting that this receptor may act as a positive regulator of the intrarenal RAS.[40,44] Augmented renin staining in the CD by Ang II and in streptozotocin-induced diabetes mellitus has been reported.[19,36,45] In rats with Ang II–dependent hypertension with marked suppression of PRA, urinary levels of renin were increased which was taken as evidence of possible secretion by CD cells into the luminal fluid.[7] Renin mRNA can be detected not only in the proximal tubules (PT) but also in the CDs.[4,45] The finding that renin mRNA can be detected in renal medulla, which is devoid of JGA, would be consistent with the notion that renin secreted in CDs could be a source of urinary renin.[45] These findings, moreover, could be interpreted to suggest that renin originating from tubular sites can foster kidney RAS activation.[19,45] In this scenario, increased tubular renin could be reflected by increased urinary renin and be used as a potential marker of increased kidney RAS activity. This would be of interest particularly in the context of DKD where levels of PRA are usually low.[27,28]

The objective of the present study was, therefore, to examine the origin of urinary renin both in patients with type 1 diabetes mellitus with or without DKD and in mice with streptozotocin-induced diabetes mellitus. The role of proximal tubular reabsorption and tubular formation of renin as a potential source of urinary renin were explored in the setting of DKD by infusing recombinant renin when proximal tubular reabsorption was blocked by L-lysine. Finally, we used a renin-reporter mouse model (Ren1d-Cre;mT/mG) made diabetic using streptozotocin to examine whether the distribution of cells of the renin lineage was altered in a chronic diabetic environment.

Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Cohort

People with type 1 diabetes mellitus, who were seen in the Diabetes Care Center (University of Washington) for outpatient endocrinology care, were approached for participation in the Kidney Research Institute Diabetic Kidney Disease Repository (University of Washington) and were enrolled into the repository after providing informed consent. Demographic and clinical information was obtained from the electronic medical records and a questionnaire completed by the participants. DKD was defined as either a urine albumin-to-creatine ratio (ACR) ≥300 mg/g or an estimated glomerular filtration rate (GFR) <60 mL/(min·1.73 m^2) and ACR ≥30 mg/g. People with longstanding diabetes mellitus but no evidence of overt DKD were those with ≥25 years of type 1 diabetes mellitus, estimated GFR ≥90 mL/(min·1.73 m^2), and ACR <30 mg/g. Demographic and clinical data (age, race, sex, diabetes mellitus duration, and RAS inhibitor use) were obtained from the electronic medical records and confirmed by patient questionnaires (race, diabetes mellitus duration and RAS inhibitor use). Diabetes mellitus type was extracted from the clinical notes, as ascertained by the endocrinologists caring for the patients. Hemoglobin A1c and serum creatinine were obtained from the electronic medical records at a time closest to the date of urine sample collection. GFR was calculated from the serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration formula. A random clean-catch, mid-stream urine sample was collected and stored at 4°C after collection, until processed The use of human samples, and data were approved by the Institutional Review Board of the University of Washington. Urine samples used for this study were centrifuged at 4700*g* for 15 minutes at 4°C, and the supernatant was collected, aliquoted, and stored at −80°C. The mean (SD) time from sample collection to storage at −80°C was 5.7 (2.0) hours.

Laboratory Measurements in Human Urine Samples

Albumin and creatinine were measured using an immunoturbidometric assay and the modified rate Jaffe reaction, respectively, using a DXC 600 clinical chemistry analyzer (Beckman Coulter, Indianapolis). Active renin was measured using a quantitative solid-phase sandwich ELISA distributed by DRG Instruments (Marburg, Germany) with a minimum detection limit of 0.8 pg/mL and 0.69% crossreactivity with prorenin. Before active renin measurements, each sample was individually concentrated (6×) using Spin-X 5 kD devices. The coefficients of intra and interassay variation for active renin ELISA in human urine samples were 4.9% and 12.7%, respectively. Internal controls were included (one for high renin and one for low renin values) to assure consistency of interassay performance. In addition, ELISA data for urine active renin were compared with those reported in the literature. The geometric mean (range) in our study for all tested urines with detectable active renin were 1.64 (0.14–17.2) pg/mL (n=65). This was in keeping with the geometric mean and range reported in the literature (1.46 (0.13–157) pg/mL using a completely different approach (enzyme-kinetic assay) in 43 urines from mainly type 2 diabetic subjects.[46]

In addition, total renin was measured using a quantitative solid-phase sandwich ELISA distributed by R&D Systems (Minneapolis, MN) with a sensitivity of 14.8 pg/mL. According to the manufacturer, this assay recognizes both human recombinant prorenin and renin. The kit for total human renin was tested by the manufacturer specifically for human urine samples.

We also attempted to measure urinary prorenin using a quantitative solid-phase sandwich ELISA distributed by Molecular Innovations (Novi, MI) with a minimum detection limit of 0.016 ng/mL. This assay was reported by the manufacturer to be specific for prorenin only with no detectable crossreactivity with human, mouse, and rat renin. However, because of poor reproducibility of prorenin measurements in our samples (Interassay coefficient of variation=69.5%, n=47), we do not present the data obtained with human prorenin assay as we had to deem the results as questionable.

Experimental Animals

Female Friend Virus B NIH Jackson mice were acquired from Jackson Laboratories (Bar Harbor, ME).

The *Ren1d-Cre; mT/mG* is a double transgenic reporter mouse model expressing Cre recombinase from the endogenous renin locus (*Ren1D*) and the *mT/mG* cassette from the *Rosa26* locus.[10] The 2 reporter genes, membrane-targeted fluorescent tomato protein (mT, red) and membrane-directed eGFP (enhanced green fluorescent protein; mG) were used to trace renin lineage cells (RLCs) in kidneys of diabetic and control mice. Whereas mT is expressed before recombination, mG is only expressed in cells post–Cre-expression and recombination. Thus, because gene expression from the *Rosa26* locus is ubiquitous and gene switch is stably transferred to cell progeny, only RLCs are green (mG positive), whereas all non-RLCs remain mT positive. These reporter mice were generated as previously described.[6,47]

Animals were housed at the Center for Comparative Medicine at Northwestern University Feinberg School of Medicine. Animal care and procedures were approved by the Institutional Animal Care and Use Committee at Northwestern University and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the institutional, state, and federal guidelines. The total number of animals enrolled was 126.

Diabetes Mellitus Induction Using Streptozotocin

Streptozotocin (150 mg/kg, Sigma Chemical, St Louis, MO) dissolved in sodium citrate buffer pH 4.5 (streptozotocin-treated mice) or sodium citrate buffer pH 4.5 alone (nondiabetic vehicle controls) was injected in 2 intraperitoneal injections to female FVB mice at 19 to 20 weeks of age. Spot urines were collected 8 and 20 weeks after the last streptozotocin or vehicle injection (at 28 and 40 weeks of age, respectively) and stored at −80ºC. Human recombinant renin (hrRenin, Molecular Innovations, Novi, MI) was administered as a single intravenous bolus injection to female FVB/N mice at the dose of 0.5 µg/g body weight. To determine the impact of renin reabsorption in the proximal tubule, lysine, a blocker of proximal tubular reabsorption,[48] was administered (0.4 mg/g body weight) as a single combination injection together with hrRenin and as a single injection without hrRenin. Urine was collected immediately before (baseline) and within 3 hours after injection. The timeline of the experiment was as follows: mice were weighted; then baseline urines were collected; within 5 to 10 minutes after voiding, mice were administered with lysine, hrRenin, or both in a single intravenous injection (0.2 mL/mouse); immediately after intravenous injection to collect urine, mice were placed for 3 hours in urine collection cages with access to water and food.

Laboratory Measurements in Mouse Samples

Ang I and Ang II were extracted and measured by Liquid Chromatography - Tandem Mass Spectrometry as previously described.[49] Active human renin was measured using a quantitative solid-phase sandwich ELISA distributed by DRG Instruments (Marburg, Germany). Mouse total renin was measured using a quantitative solid-phase sandwich ELISA distributed by RayBiotech (Norcross, GA) with a minimum detection limit of 6 pg/mL. Albumin was measured by a quantitative solid-phase sandwich ELISA (Albuwell M, Exocell, Philadelphia, PA). Creatinine was measured using the Jaffe method (Creatinine Companion, Exocell, Philadelphia, PA).

Western Blot of Mouse Urine Samples

For Western blot of urines, proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked in nonfat dry milk solubilized in Tris-buffered saline solution, pH 8.0 containing 0.1 %Tween 20 (7% wt/vol). The PDVF membranes were incubated with primary anti-Renin/Prorenin antibody (Molecular Innovations) and horseradish peroxidase–conjugated secondary antibody (Santa Cruz Biotechnology). Bands were visualized using chemiluminescence system (Super Signal Pico, Pierce). For comparisons of urinary renin bands between control and lysine-injected groups, several gels were used in which a similar number of samples from each group was included. The average value of integrated density measured in controls was set as 100% for each gel, and the results for injected mice were expressed as percentage of that measured in controls.

Immunohistochemistry

Paraffin blocks were cut at 4 µm and deparaffinized in xylene and rehydrated through graded alcohols. Antigen retrieval was performed with a pressure cooker at 120°C in target retrieval solution (DAKO). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide. Slides were incubated overnight at 4°C with rabbit anti-mouse renin polyclonal antibody (1:500), washed, and incubated with secondary antibody conjugated with peroxidase-labeled polymer (DAKO). After incubation with 3,3′-diaminobenzidine chromogen, slides were counterstained with hematoxylin. Sections were dehydrated and covered with Permount (Fisher Scientific). Coverslips were viewed using a Zeiss microscope. To evaluate renin protein expression in kidneys from streptozotocin-treated and vehicle control mice (n=9 in each group), a semiquantitative analysis of the immunoperoxidase-stained sections was performed. Two masked observers performed evaluation of renin staining within JGA, CD, and PT. The percentage of JGAs with visible renin staining, the number of renin stained collecting tubules, and the intensity of PT staining in the viewing areas (5 viewing fields per slide) were evaluated. The percentage of renin-positive JGA (JGA index) was calculated as (the number of renin-positive JGA in all sections)×100/(the total number of glomeruli observed).[50] Every section was examined observer-blinded using the same magnification (×200), and only the JGA with a classic donut-shaped outline was evaluated.

Immunofluorescence

Cryosections (7 µm) were cut using a cryostat from the frozen blocks of Ren1d-Cre; mT/mG mouse kidneys and subsequently subjected to the immunostaining for AQP2 (aquaporin 2; 1:200; Santa Cruz Biotechnology) and renin. Kidney sections from Ren1d-Cre;mT/mG mice were screened for the presence of GFP+ in CD epithelial cells based on colocalization with AQP2 immunostaining in both diabetic and nondiabetic animals using Fiji software (National Institutes of Health). A Pearson correlation coefficient was used to quantify the colocalization parameters. Moreover, a large image analysis of the GFP and AQP2 colocalized area per total area was performed to assess any differences between kidneys of control and diabetic Ren1d-Cre;mT/mG mice.

In addition, kidney sections were assessed for the presence of GFP+ cells in control and diabetic Ren1d-Cre;mT/mG mice within the parietal glomeruli and glomerular tuft and quantified as percentage of glomerular GFP positivity.

Microdissection of Renal Tubule Segments

Streptozotocin-treated mice and vehicle controls were euthanized by an overdose of Euthasol (390 mg pentobarbital-Na and 50 mg phenytoin-Na per mL).

Both kidneys were rapidly removed and transferred into ice-cold HEPES solution. After removal of the capsula and pelvis, the kidneys were cut into 1- to 2-mm thick coronal slices. Usually, 3 or 4 slices from the middle of each kidney could be obtained due to the thickness of the cortex and medulla. For the preparation of the tubules, 1- to 2-mm slices of the respective region were prepared under a stereo microscope and transferred into the prewarmed digestion solution. Cortical and outer medullary tissue was then incubated in a digestion solution containing 4 mL MEM (GibcoBRL), 5 mmol/L glycine, 6 mg/mL trypsin inhibitor type II-S (Sigma T-9128), and 250 g/mL collagenase (Sigma C-9891), pH 7.4 at 37ºC in a water bath for 25 minutes without shaking. When the medium became cloudy on gentle shaking, the digestion was stopped by transferring the tubules to ice, carefully removing the supernatant and replacing it with 4 mL ice-cold 1% BSA-HEPES solution. The BSA-HEPES solution was replaced with ice-cold HEPES solution after 10 minutes and tubules were maintained on ice until use. Isolated tubules were selected at 4ºC under a stereo microscope after 0.5 mL of the tubule suspension had been diluted in 10 mL of ice-cold HEPES solution. Individual tubule segments were identified by their appearance and topology. For the proximal tubule segments, S1 was identified as the proximal tubule directly attached to the glomerulus, S2 was the straight part in the medullary ray, and S3 was the proximal tubule in the outer medulla. The cortical CD (CCD) was dissected from the medullary rays of the cortex. Around 100 proximal tubule segments and 30 CCD segments were dissected from each mouse kidney.

Reverse Transcription–Polymerase Chain Reaction Analysis

Total RNA from kidney cortex tissue was obtained by using Trizol reagent (Thermo Fischer Scientific, Waltham, MA). Constant amounts of 1 µg of extracted kidney cortex RNA, as well as the totality of the total RNA obtained from microdissected tubules (using Arcturus PicoPure RNA kit from Applied Biosystems), were reverse transcribed to synthesize cDNA. Synthesis of cDNA was performed using Reverse Transcription Kit on a GenAmp PCR System 9700 (both Thermo Fischer Scientific, Waltham, MA) with standard cycling parameters.

Quantitative polymerase chain reaction was run on a 96-well plate using a Step One Plus PCR System (Thermo Fischer Scientific, Waltham, MA). GAPDH was used as an internal control for normalization. Each reaction was performed in duplicates. Sequences of the primers for quantitative polymerase chain reaction (IDT, Coralville, IA) are shown in Table S1 in the online-only Data Supplement.

Statistical Analysis

Shapiro-Wilk was used to test normality. Normally distributed data are reported as the arithmetic mean±SEM. Distribution of nonnormally distributed data was described using median and interquartile range. Differences between 2 groups with normal distribution were analyzed using a 2-tailed Student *t* test and for nonnormally distributed data (eg, urinary ACR), using nonparametric Mann-Whitney test. To assess correlation between variables, Spearman correlation coefficient was used for nonnormally distributed data and Pearson correlation coefficient for normally distributed data. Fisher exact test was used in the analysis of contingency tables. Interaction between 2 independent variables was assessed using 2-way ANOVA. The nominal significance threshold was set as a 2-sided $P<0.05$. The IBM SPSS software (version 23) and GraphPad Prism software (version 8) were used for the statistical analyses.

Results

Studies in Urine Samples From Patients With Type 1 Diabetes Mellitus

Characteristics of the Cohort of Patients With Type 1 Diabetes Mellitus

Urine samples were obtained from a carefully characterized cohort of patients with type 1 diabetes mellitus at the University of Washington, Seattle. The majority of patients were white in both groups. There were no significant differences in age and sex distribution between patients with and without DKD, which both had male predominance (Table S2). Hemoglobin A1c was significantly higher in the DKD than in the non-DKD group (median 8.3 versus 7.5%; $P=0.002$). Both groups had prolonged disease duration of diabetes mellitus, but it was slightly but significantly longer in the group without DKD than in the group with DKD (median 32 versus 37 years, $P=0.032$). Therefore, the group without DKD had very low risk of developing it after such a long period of disease duration.[51]

As expected from the selection criteria, the group without DKD had urinary albumin excretion in the normal range and an estimated GFR above 90 mL/(min·1.73 m^2), whereas the group with DKD had urinary albumin excretion either in the micro or macroalbuminuric range and an estimated GFR below 60 mL/(min·1.73 m^2). The median estimated GFR was lower (median 39 versus 101 mL/[min·1.73 m^2]; $P<0.001$), whereas ACR was higher (median 497 versus 7 mg/g creatinine; $P<0.001$) in people with DKD, compared with those without DKD (Table S2). RAS-blocker use was more prevalent in people with DKD than in those without (86 versus 50%; $P=0.001$).

Urine Renin and Albumin in Patients With and Without DKD

Active urinary renin was detectable in 94% (34/36) of urines from the cases and 82% (31/38) of urines from controls with no DKD. Total urinary renin was detectable in 56% (20/36) of samples from the cases but only in 9% (5/35) of the urines from the controls with diabetes mellitus but no DKD. For subsequent analyses, the values below the lower limit of quantification of the assays were set to 0.5×lower limit of quantification. Median creatinine-normalized urine active renin concentrations were significantly higher in people with DKD as compared to those without DKD (3.2; 2.1, 9.5 versus 1.3; 0.5, 2.8 pg/mg creatinine; $P<0.001$; Figure 1A). The urinary albumin/creatinine ratio was markedly higher (by study design) in the group with CKD than in the group without (Figure 1B). A significant positive correlation between active renin and ACR was found (Spearman coefficient: $r=0.454$, $P=0.000062$, 95% CI, 0.2420–0.6247).

Median creatinine-normalized urine total renin concentrations were also significantly higher in people with DKD than in people without DKD ($P=0.023$; Figure S1). A positive correlation between creatinine-normalized total urine renin and creatinine-normalized urine albumin was found (Spearman coefficient: $r=0.410$, $P=0.0005$, 95% CI, 0.1851–0.5941).

Urinary Mouse and Plasma Renin in Experimental DKD Caused by Streptozotocin in Mice

At 8 weeks post-streptozotocin injections, a significant increase in urinary renin normalized for creatinine was found in mice treated with streptozotocin (n=15) compared with those treated with vehicle (n=8; 455±106 versus 113±27 pg/mg creatinine; $P=0.005$) (Figure 2A). Albumin was significantly increased in mice injected with streptozotocin compared with controls (69±23 versus 11±3 µg/mg creatinine; $P=0.006$) as was the blood glucose (549±15 versus 157±6 mg/dL; $P<0.01$). Twenty weeks after streptozotocin administration, hyperglycemia and albuminuria also developed, and the data on these parameters have been previously reported.[23] Here, we show urinary data from the same animals, not previously reported. Urinary total mouse renin/creatinine ratio was significantly higher in mice treated with streptozotocin (1093±319 pg/mg creatinine, n=15) as compared to those treated with vehicle (64±19 pg/mg creatinine, n=8, $P=0.0001$; Figure 2B). A significant positive correlation was found between total renin and albumin both at 8 (Spearman coefficient: r=0.477, $P=0.018$, 95% CI, 0.07867 to 0.7441) and 20 poststreptozotocin injection (Spearman

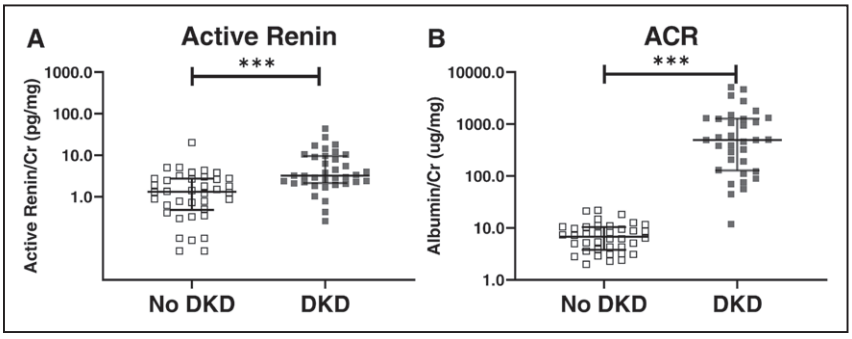

Figure 1. Active renin/creatinine (Cr) ratio and albumin/Cr ratio in patients with and without diabetic kidney disease (DKD) in longstanding type 1 diabetes mellitus. People with no DKD had estimated glomerular filtration rate (eGFR) ≥90 mL(min·1.73 m^2) and albumin-to-Cr ratio (ACR) <30 mg/g after ≥25 y of type 1 diabetes mellitus. Those with DKD had either an ACR ≥300 mg/g or both eGFR <60 mL(min·1.73 m^2) and ACR ≥30 mg/g. **A**, higher active renin/Cr ratio in urines from patients with DKD (black, n=36) compared with those without DKD (white, n=38). **B**, Higher albumin/Cr ratio in urines from patients with DKD (black, n=34) compared with those without DKD (white, n=38). *** $P<0.001$.

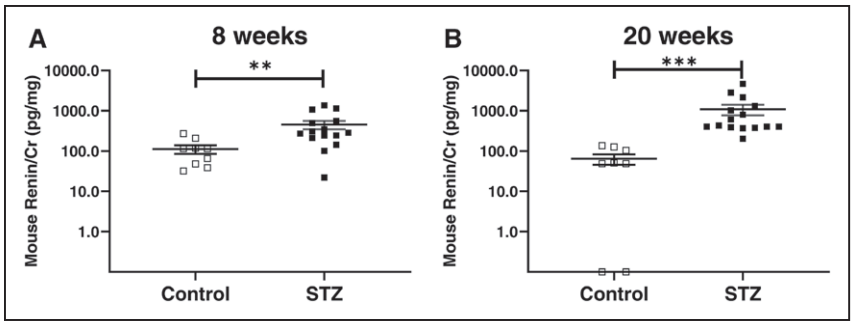

Figure 2. Mouse renin concentrations in nondiabetic and diabetic mice, 8 (**A**) and 20 wk after the last streptozotocin (STZ) injection (**B**). **A**, higher mouse renin/creatinine (Cr) ratio in urines from diabetic FVB mice (STZ, black, n=15) compared with nondiabetic FVB mice (Veh, white, n=9) 8 wk after the last injection of steptozotocin. **B**, Higher mouse renin/Cr ratio in urines of diabetic FVB mice (STZ, black, n=15) compared with nondiabetic FVB mice (Veh, white, n=8) 20 wk after the last injection of STZ. Data shown as mean±SE. STZ denotes streptozotocin-treated mice. Veh indicates control mice treated with vehicle; $^{**}P<0.01$, $^{***}P<0.001$.

coefficient: r=0.704, P=0.0002, 95% CI 0.4006–0.8685), respectively. When the 2 age groups were combined, a significant positive correlation was also found Spearman coefficient: r=0.628, P=0.0000023, confidence interval 0.4089–0.7790).

Because we did not have enough plasma volume for PRA measurements, we assessed indirectly using an approach recently described by Domenig et al.[52] In this approach, the summation of Ang I and Ang II serves as a strong surrogate for PRA.[52] We applied this concept to our studies and thus inferred PRA from the sum of Ang I and Ang II measured by LC-MS/MS in streptozotocin-treated and control mice. The inferred PRA from the sum of plasma Ang I and Ang II was lower in streptozotocin injected mice than in the control group, but the difference was not statistically significant (83±52 versus 267±179 pg/mL; P=0.230).

Effect of L-lysine Infusions on Urinary Renin

The effect of lysine on total urinary renin excretion was first evaluated in a separate set of control mice using Western blots. A renin immunoreactive protein band appeared at around 37 kDa in urines from mice injected with L-lysine (n=6), which was barely detectable in urines of noninjected control mice (n=7; Figure S2A). Densitometric analysis showed that the relative abundance of the renin immunoreactive protein band at 37 kDa was significantly increased in urines from mice injected with lysine compared with urines from noninjected controls (1912±890 % of control FVB mice, P=0.001; Figure S2B).

Having shown the increase in renin protein by Western blot, we next used ELISA. Urinary creatinine-normalized renin was >100-fold increased in mice injected with lysine (n=7) compared with noninjected controls (n=8; 8950±2337 versus 64±19 pg/mg creatinine; P=0.001; Figure 3A). This data shows that lysine, by blocking proximal tubular reabsorption of endogenous renin, results in a large increase of urinary renin.

In diabetic mice, urinary renin/creatinine ratio in mice injected with lysine (n=8) was about 10-fold increased as compared to noninjected mice (n=15; 11 372±4126 versus 1093±319 pg/mg creatinine; P<0.001, Figure 3A). Although the levels of renin were higher in diabetic mice than in controls, the relative renin/creatinine increase after lysine injection is actually greater in control mice than in diabetic mice. This suggests a preexisting defect of proximal tubular reabsorption in diabetic mice. In noninjected mice, urinary renin/creatinine ratio is significantly increased in diabetic mice compared with control mice (1093±319 versus 64±19 pg/mg creatinine; P<0.001), whereas in lysine-injected mice, the increase of renin/creatinine ratio in diabetic mice compared with control mice no longer is significantly different (11 372±4126 versus 8950±2337 pg/mg creatinine; P=0.908, Figure 3A). Moreover, the effect of lysine administration was not significantly different between streptozotocin-treated and control mice when analyzing the interaction between lysine and diabetes mellitus status by 2-way ANOVA (P=0.4).

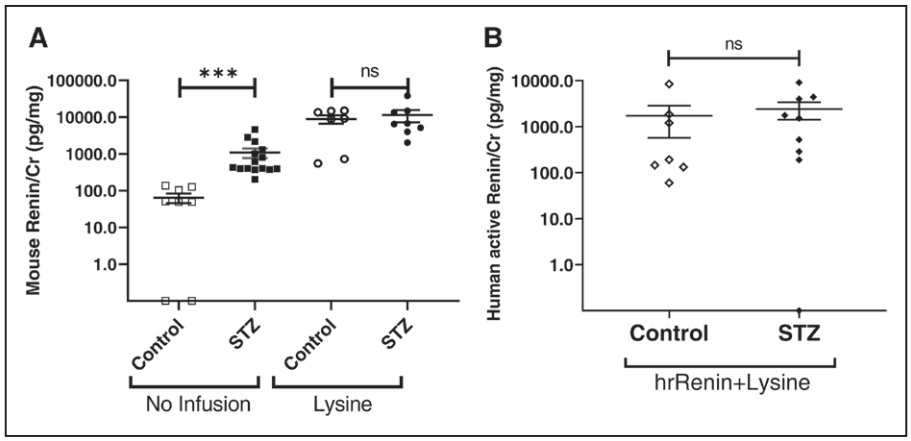

Figure 3. **A**, Effect of lysine on urinary mouse renin in control and diabetic mice (**B**). Human active renin/creatinine (Cr) in nondiabetic and diabetic mice infused with human recombinant renin (hrRenin) and lysine. **A**, Mouse renin/Cr ratio in urines from control (white) and diabetic (black). Mice were infused with lysine (Lysine [circles]; control n=7, diabetic n=8) or did not receive lysine infusion (no infusion [squares]; control n=8, diabetic n=15). In noninfused animals, total renin was higher in streptozotocin (STZ)-treated compared with controls, whereas after lysine infusion no significant differences existed between the 2 groups. Data shown as mean±SE. $^{***}P<0.001$. **B**, In mice infused with both hrRenin and lysine, urinary human active renin/Cr is markedly elevated, but there is no significant difference in STZ treated (closed symbols) vs nondiabetic mice (control, open symbols). Human active renin is undetectable in urines from mice infused with lysine only or from mice not infused with hrRenin (not shown, see text). Data shown as mean±SE.

Urinary Human Active Renin Concentrations After Human Recombinant Renin and Lysine Infusions to Normal Mice and Streptozotocin-Treated Mice

hrRenin was injected to control and streptozotocin-treated mice to examine the renal handling of exogenous renin, distinct from mouse renin. In this model, the renin recovered in the urine provides an estimate of the renal handling of exogenous renin. As expected, human active renin was not detectable in urine samples from noninfused mice and mice infused with lysine only. This shows that the ELISA for human active renin does not recognize mouse renin. Urines of control mice injected with hrRenin (n=12) showed substantial levels of creatinine-normalized human active renin as compared to noninfused control mice in which it was not detectable (n=17; 30±11 versus 0±0 pg/mg creatinine; $P<0.001$). This demonstrates that human renin can be filtered but does not reveal the extent of how much is reabsorbed. In diabetic mice, injection of hrRenin (n=10) also resulted in an increase in urinary creatinine-normalized-human active renin as compared to noninjected diabetic mice (n=15; 779±625 versus 0±0 pg/mg creatinine; $P<0.001$).

The difference in urinary hrRenin between control and diabetic mice after infusion of hrRenin was large (779±625 versus 30±11 pg/mg creatinine, $P=0.032$). This reflects either increased filtration, impaired tubular reabsorption or both in diabetic mice.To estimate the effect of tubular reabsorption on urinary renin, lysine was infused together with hrRenin. Urines of control mice injected with a combination of lysine and hrRenin (n=7) had markedly higher urinary creatinine-normalized hrRenin than those of control mice infused with hrRenin only (n=12; 1720±1151 versus 30±11 pg/mg creatinine, $P=0.001$). In the streptozotocin-treated group, combined injection of lysine and hrRenin (n=9) also resulted in an increase of urinary human active renin compared with injection with hrRenin only (n=10), but it did not reach statistical significance (2418±994 versus 779±625 pg/mg creatinine, $P=0.05$). There was no apparent interaction between injection of lysine and diabetic status when analyzed by 2-way ANOVA ($P=0.3$).

In the streptozotocin-treated group infused with a combination of hrRenin and lysine, urinary human active renin was not significantly different than in controls infused with a combination of hrRenin and lysine (2318±994 versus 1720±1151 pg/mg creatinine, $P=0.368$, Figure 3B). Thus, in the presence of lysine induced blockade of proximal tubule reabsorption, the amount of infused human renin that appears in the urine is about the same in diabetic mice as in controls. This suggests the preexistence of a defect in proximal tubular reabsorption of renin in diabetic mice.

Kidney Renin Immunostaining in Streptozotocin-Treated Mice

Immunohistochemistry was used to examine renin protein expression in the kidney from control and streptozotocin-treated mice. By immunohistochemistry, the main sites of staining were the JGA and kidney collecting tubules. Control animals had strong JGA staining and weak renin staining in collecting tubules. In contrast, in kidneys from streptozotocin-treated animals, JGA staining was reduced, whereas collecting tubule (CD) staining was increased (Figure 4A and 4B). The differences in JGA and CD staining between control and streptozotocin-treated mice was statistically significant ($P<0.01$) based on observations performed by 2 blinded independent observers. Renin staining was also observed along the brush border of PT, but it seemed to be much weaker than in JGA and in CD. Based on observations performed by 2 blinded independent observers, PT renin staining in diabetic mice was significantly less abundant than in control mice (0.59±0.08 versus 1.09±0.09 average intensity of renin stained PT/area; $P=0.0012$; Figure 4C).

Kidney Renin mRNA Expression in Kidney Cortex and Microdissected Tubules

Renin/GAPDH mRNA in the kidney cortex, which is largely representative of juxtaglomerular mRNA levels, was significantly lower in diabetic (n=12) as compared to control mice (n=11; 0.78±0.1 versus 1.39±0.3, $P = 0.04$, Table S3). We next investigated whether the decreased renin staining in the PT and increased renin staining in the CD of diabetic mouse kidneys could be a result of different levels of renin mRNA expression in the respective areas. For this, we used microdissected kidney PT and CCDs from control and diabetic mice. No significant differences were observed in the levels of mouse renin mRNA in either the PT or in the CCD (Table S3).

Kidney Megalin and Mannose-6-Phosphate Receptor mRNA in Experimental DKD

To evaluate receptors that are involved in the reabsorption of renin in the proximal tubule, the mRNA of megalin and M6PR (mannose-6-phosphate receptor) was evaluated. Both receptors are present in the proximal tubule and bind renin and other proteins.[53] Reverse transcriptase real-time polymerase chain reaction was used to measure megalin and M6PR expression in kidney cortex from control and streptozotocin-treated mice. Mouse megalin mRNA in diabetic mice was detected at a significantly lower level than in control mice in kidney cortex (ratio megalin/GAPDH mRNA, 0.70±0.09 versus 1.01±0.04, $P=0.01$, n=6; Figure S3A). The levels of M6PR mRNA in diabetic mice, however, were not different from control mice in kidney cortex (ratio M6PR/GAPDH mRNA, 1.03±0.06 versus 0.97±0.07, $P=0.573$, n=6; Figure S3B).

Ren1d-Cre;mT/mG With Streptozotocin-Induced Diabetes Mellitus

A double transgenic mouse model was used to trace RLC in control and diabetic kidneys.[6] The mT/mG construct switches from membrane-directed fluorescent tomato protein (mT, red) to membrane-directed eGFP (mG) expression after Cre-mediated recombination. Therefore, all RLCs are mG positive, whereas all non-RLCs remain mT positive.[6] Ren1d-Cre;mT/mG was made diabetic using streptozotocin, and the animals were studied 11 to 12 weeks after the last streptozotocin injection.

To examine the RLCs in the CD, immunostaining for AQP2 was used as a marker of principal cells. Using confocal laser scanning microscopy, we analyzed the overlay of mG positive RLCs and AQP2 positive CD cells. Colocalization was quantified using Fiji software. We found no significant differences in colocalization at CD sites between kidneys from diabetic (n=5) and control mice (n=5; Pearson correlation coefficient: 0.34±0.04 versus 0.31±0.04; $P=0.632$; Figure 5). This finding shows that the increased renin staining noted above using

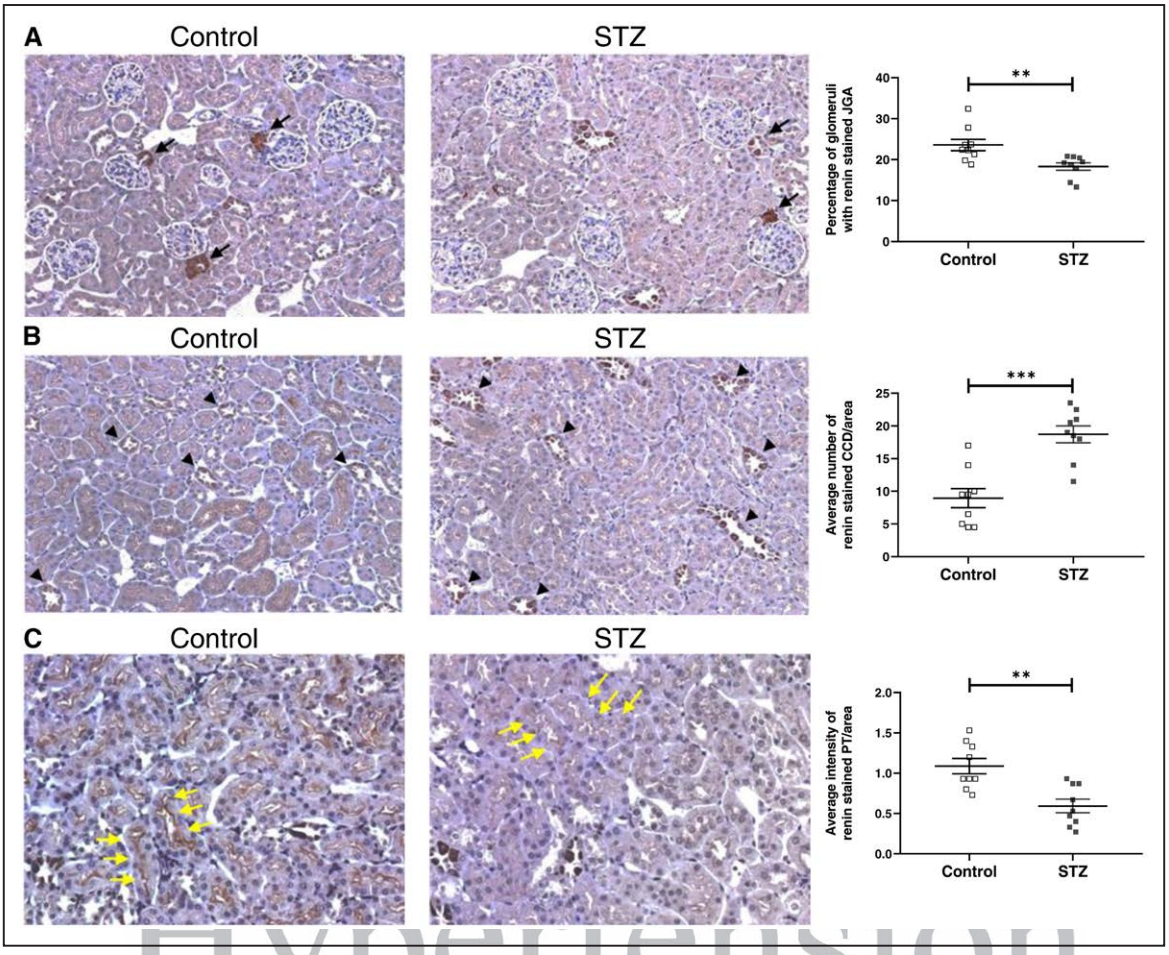

Figure 4. Kidney renin immunostaining in nondiabetic and diabetic FVB mice and semiquantitative analysis of staining in the juxtaglomerular apparatus (JGA; **A**), cortical collecting duct (CCD; **B**) and proximal tubule (PT; **C**). **A**, Immunostaining for renin shows a significant decrease of the percentage of glomeruli with stained JGA (black arrows) in diabetic FVB kidneys (streptozotocin [STZ]) compared with nondiabetic FVB kidneys (control). **B**, Immunostaining for renin shows a significant increase in the average number of stained CCD per area (black tip) in kidneys of diabetic FVB mice compared to nondiabetic FVB mice. **C**, Immunostaining for renin shows a significant decrease in the average intensity of stained PT per area (yellow arrow) in diabetic FVB kidneys compared with nondiabetic FVB kidneys. STZ denotes streptozotocin-treated mice.

immunohistochemistry (see Figure 4) cannot be attributed to the migration of RLCs to the CD in diabetic mouse kidneys.

We next evaluated the RLCs in the glomeruli of diabetic and nondiabetic renin-reporter mice. In both, mG positive cells were found within the Bowman capsule of the glomerulus and to a lesser extent in the intraglomerular compartment namely, mesangial cells (see examples in Figure S4A). The quantitation of the frequency mG positive cells revealed that in the parietal epithelium of the glomeruli RLC cells were observed in about 40% of the time, whereas within the intraglomerular mesangium, they were found in only 5% to 10% of the time (Figure S4B). Comparing control and streptozotocin-treated mice in regards to mG positive cells, no significant differences were found in either parietal or intraglomerular staining between both groups (Figure S4B).

Finally, renin protein staining was performed in renin-reporter mice to find out which of the RLCs are associated with renin protein. Positive colocalization of renin with RLC near the vascular pole of the glomerulus was found, reflecting the physiological site of renin secretion (Figure 6A). By contrast, only few tubular epithelial structures had a colocalization of renin and RLC (Figure 6B).

Discussion

This report shows that in people with type 1 diabetes mellitus who have developed DKD, urinary renin is increased as compared to a group who had not developed it after similar disease duration (≥25 years of diagnosis of diabetes mellitus). Consistent with the findings in humans with DKD, urinary renin was also markedly increased in streptozotocin-treated diabetic mice, a model of early DKD. The concordant increase in urinary renin in an experimental model of diabetes mellitus and in human DKD associated with type 1 diabetes mellitus is in agreement with previous studies where key components of the RAS system including renin were similarly altered.[23,46,54]

Renin-producing cells in adult life are restricted to the JGA localization, whereas in fetal life these cells are also found in large intrarenal arteries, inside the glomeruli, and in the interstitium.[6] As maturation continues, the number of renin-producing cells is reduced as they differentiate into arteriolar smooth muscle and mesangial cells and become progressively restricted to the JGA localization.[5,6]In response to a threat to homeostasis, requiring more renin, smooth muscle cells, and mesangial cells may acquire the renin phenotype and synthesize renin again until the crisis passes.[5,6] For instance, agents that inhibit the response

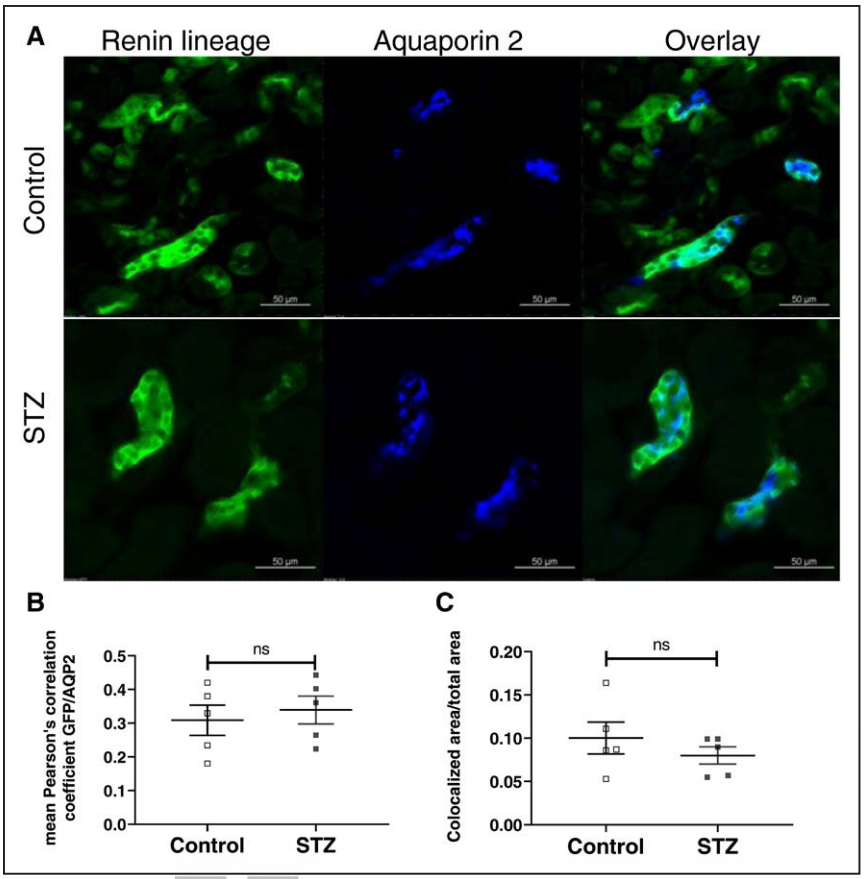

Figure 5. Renin lineage and aquaporin 2 expressing cells in nondiabetic and diabetic Ren1d-Cre;mT/mG mouse kidneys (**A**) and colocalization analysis (**B**, **C**). **A**, Kidney sections from Ren1d-Cre;mT/mG mice show the presence of GFP (green fluorescent protein)+ collecting duct epithelial cells. Immunofluorescence for AQP2 (aquaporin 2) show colocalization with GFP+ collecting duct cells in both diabetic and nondiabetic Ren1d-Cre;mT/mG mice. **B**, Colocalization analysis of GFP+ cells and immunofluorescence for AQP2 cells using Pearson correlation coefficient show no difference between kidneys of control and diabetic Ren1d-Cre;mT/mG mice. **C**, A large image analysis of the GFP and AQP2 colocalized area per total area shows no significant difference between kidneys of control and diabetic Ren1d-Cre;mT/mG mice. STZ denotes streptozotocin-treated mice; n=5 in each group. ns indicates nonsignificant.

to Ang II (such as the ACE [angiotensin-converting enzyme] inhibitor captopril) result in an increase in the number of JGA cells that express renin and an accompanying increase in the number (recruitment) of renin-expressing cells along the afferent arteriole and sometimes in the glomerulus.[9] By contrast, ureteral reflux leads to recruitment of renin-expressing mesangial cells and tubular cells.[55] Despite its importance as the most frequent cause of CKD, the impact of diabetes mellitus on RLC has not been previously studied. To ascertain whether there is an increase in RLC along the nephron and particularly in the CD that could

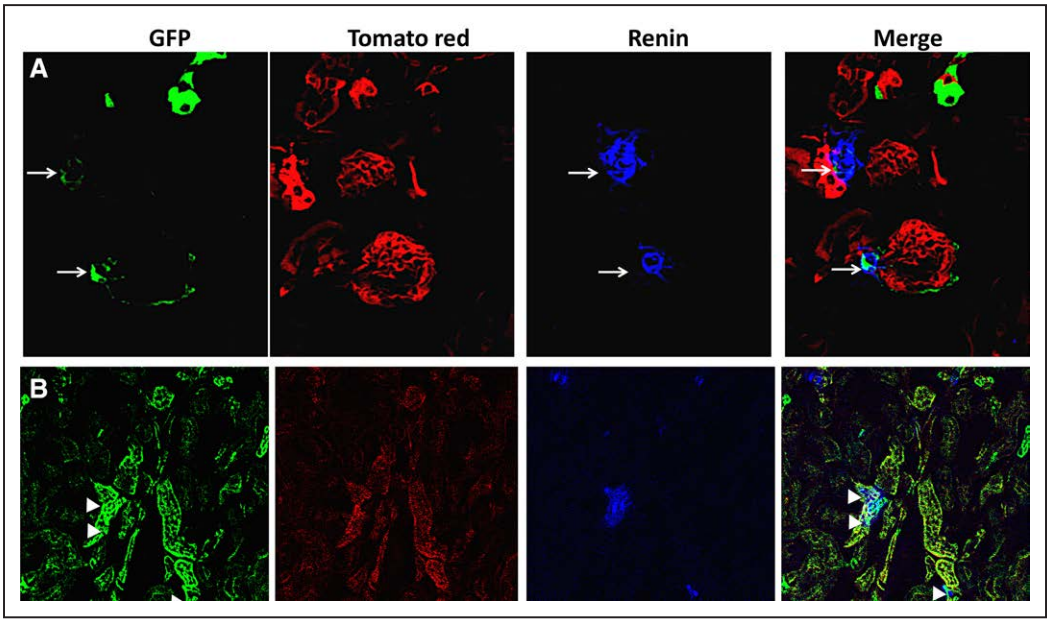

Figure 6. Renin colocalization with renin lineage cells (RLC) near the glomerular pole (**A**, arrows) and in tubules (**B**, arrowheads) in kidney sections from renin-reporter mice. Green reflects RLC and tomato red labels the kidney structures. Renin protein staining (blue). The areas of colocalization in turquoise color of renin and renin lineage cells are near the glomerular pole (merged image in **A**, white arrows) and in few areas of tubular epithelium (merged image in **B**, arrowheads). GFP indicates green fluorescent protein.

account for increased urinary renin production in streptozotocin-treated mice, we developed a mouse line resulting from the cross of *Ren1d-Cre* mice with *mT/mG* reporter mice and used streptozotocin to induce chronic diabetes mellitus.[6] These mice, GFP labels cells (and all their descendants) permanently at the sites where Cre-recombination occurs, enabling tracing of cells of renin lineage, that is, those cells that either actively express renin or are derived from ureteric bud epithelial cells or Six 2 derived progenitors that expressed renin earlier during development.[6]

Consistent with previous studies,[6] we found that in *Ren1d-Cre;mT/mG* mice the GFP+ cells were present in the renal arterial tree (including JGA cells), CD epithelial cells in the kidney cortex, medulla, and papilla. Immunostaining for aquaporin colocalized with GFP+ CD cells of *Ren1d-Cre;mT/mG* mice (Figure 5A). There were, however, no appreciable differences in colocalization pattern or intensity between renin-reporter mice with and without diabetes mellitus (Figure 5B). We also observed frequent expression of RLCs in the glomerular parietal epithelium and their occasional expression within the glomerular tuft (Figure S4A). The level of expression of GFP positive cells within either of these glomerular locations was not significantly different between control and streptozotocin-treated mice (Figure S4B). In the aggregate, therefore, data from the renin-reporter mice provides no evidence of migration of RLCs to the CD or intraglomerular locations in mice made diabetic by streptozotocin as compared to nondiabetic controls.

The lack of differences in the presence of RLCs at various nephron segments does not inform, however, whether the GFP+ cells at the time after diabetes mellitus induction actively produce renin or are dormant. We, therefore, evaluated renin mRNA expression in microdissected kidney PT and CCD from control and streptozotocin-treated diabetic mice. No significant differences were noted in the levels of mouse renin mRNA in the PT or in the CCD (Table S3). This suggests that local renin production at those sites cannot contribute to the markedly increased urinary renin found in streptozotocin-treated mice. In the CDs of streptozotocin-treated diabetic mice, by contrast, the renin immunostaining was increased as previously reported for prorenin.[19] The possibility that part of the increased urinary renin that we find in diabetes mellitus could originate from conversion of prorenin in the collecting tubule cannot be ruled out in our studies. In our study, total renin enzyme immunoassay, that simultaneously detects both active renin and prorenin, yielded 10–100 higher values than the active renin enzyme immunoassay (Figure 1A and Figure S1). By simple subtraction of the results of 2 assays, one could assume that in the final urine the prorenin might predominate. Against this assumption, however, is the evidence presented by Roksnoer et al[56] who demonstrated that the renin standard included in the total renin enzyme immunoassay assay causes an upward shift resulting in artificially inflated total renin values. In addition, studies in humans and rats suggest that urinary renin reflects mostly active renin and not prorenin because almost no prorenin could be detected in the final urine.[37,46]

In the renal cortex, renin mRNA was significantly reduced in streptozotocin-treated mice as compared to controls (Table S3). Most of kidney cortex renin mRNA reflects JGA, the major physiological site for renin production.[57] Consistent with this finding, renin staining in the JGA was reduced in streptozotocin-treated diabetic as compared to control mice (Figure 4). The finding of reduced JGA renin (by immunostaining) and reduced mRNA levels in the renal cortex, altogether provides, by extrapolation to humans, an explanation for the low levels of PRA usually seen in patients with diabetic complications.[26–30]In fact, DKD is the most frequent cause of the syndrome of isolated hyporeninemic hypoaldosteronism.[58–61] In this syndrome, the reduced levels of PRA may be multifactorial but sclerosis of the JGA associated with tubule interstitial fibrosis has been occasionally documented.[60] Our finding of reduced renin staining in the JGA of diabetic mice is, therefore, potentially relevant in explaining the known predisposition to the syndrome of hyporeninemic hypoaldosteronism, an important cause of hyperkalemia in patients with diabetes mellitus.[58,59]

Because we found no evidence for migration of RLC to the CD as the potential source of tubular renin, the increase in renin staining in CDs observed in this study by us and previously by others[19] deserves an alternative explanation. In general, urinary renin can originate from 3 different sources: from plasma renin that has been filtered and not completely reabsorbed by the proximal tubule, from renin that has been produced and released locally from kidney tubules, or from a conversion of plasma-derived prorenin to renin largely taking place distally in the CD where the PRR is abundant.[39,41–43] Because of the relatively small molecular size, renin is easily filterable and, of course, more in the presence of glomerular pathology due to DKD. Renin staining in the apical part of proximal tubule cells has long been believed to represent filtered and pinocytozed renin.[62] The proximal tubule is the principal site of uptake of proteins, including renin, a process which appears to be mainly megalin-mediated.[37,53] The observed decrease in proximal tubular renin staining (Figure 4C) suggests that increased urinary renin could be a result of diminished capacity of the proximal tubule to reabsorb renin. Of note, we found in diabetic streptozotocin-treated mice that kidney mRNA expression of megalin, an endocytosis receptor, was decreased (Figure S3A) which is in agreement with previous reports in streptozotocin-treated rats[63,64] and Ove-26 mice.[65] It has been shown that decreased kidney megalin expression in diabetic rats was associated with reduced reabsorption of albumin[63] and increased loss of other urinary proteins, such a transferrin.[64] Megalin's major role in proximal tubule renin reabsorption is suggested by studies using megalin knockout[53] We, therefore, surmise that the increase in urinary renin excretion in diabetic mice is due to a reduced kidney expression of megalin causing reduced proximal tubular reabsorption of renin. The lack of differences in renin mRNA between control and diabetic mice at the CCD, noted above, further shows that the source of urinary renin in DKD cannot be due to local production at this tubular site.

Our findings in the streptozotocin-model of diabetes mellitus are in full agreement with recent work by Roksnoer et al[37] who have emphasized that normally renin appears in the urine as a result of filtration and only in small amounts because proximal reabsorption retains the majority of the filtered renin. In a series of elegant experiments using urine samples from patients with Dent disease and Lowe syndrome, entities with impaired proximal tubular reabsorption of low molecular weight proteins, urinary renin was found markedly increased demonstrating the

importance of intact tubular processing.[37] In our studies, urinary albumin was increased in diabetic as compared to control mice likely as the result of increased filtration due to a disturbed filtration barrier in diabetic mice. Likewise, this glomerular alteration can explain increased renin in the urine even if renin secretion from the JGA is reduced. Although this undoubtedly contributes to high urinary renin, the finding of reduced expression of megalin in the kidney cortex in diabetic as compared to control mice suggests a likely role of impaired tubular reabsorption as a contributor to high urinary levels of renin in diabetic mice. Our approach to further demonstrate the importance of proximal tubular reabsorption of renin in diabetic mice was based on infusing renin with and without lysine, a blocker of proximal tubular reabsorption. Tubular reabsorption of peptides and proteins can be inhibited by coadministration of cationic amino acids, such as L-lysine or L-arginine.[66,67] Megalin binds proteins rich in positively charged amino acids and cationic compounds. It is believed that L-lysine saturate those binding sites and, therefore, interfere with renal reabsorption of proteins. Intravenously infused L-lysine has been previously used to inhibit tubular uptake of renin causing greatly increased urinary renin excretion.[66] Consistent with previous reports,[66,68] we found that L-lysine caused marked increases in urinary excretion of endogenous and exogenous mouse renin. While at baseline, urinary mouse renin was noticeably higher in diabetic than in control mice, after L-lysine injection, the markedly increased urinary mouse renin levels were no longer significantly different statistically between control and diabetic streptozotocin-treated mice (Figure 3A). Because endogenous mouse renin can originate from plasma or the kidney, we injected intravenous human active renin protein to trace the fate of only plasma renin that has been filtered. For this, human renin protein was infused and detected using an assay which identifies only human active renin and no mouse renin. Injection of human renin alone resulted in a detection of low levels of human active renin in the urine of control mice and a much accentuated urinary levels in diabetic mice. Similar to endogenous mouse renin, coadministration of L-lysine with human renin resulted in marked augmentation in urinary levels of active human renin in both diabetic and nondiabetic mice, such that urinary renin was no longer significantly different (Figure 3B). The findings of similar levels of both mouse (Figure 3A) and human renin (Figure 3B) when proximal tubular reabsorption is blocked by lysine shows that increased urinary renin in diabetes mellitus is largely due to impaired proximal tubular reabsorption. It should be noted, however, that L-lysine can increase the GFR, which might contribute to increased levels of renin in the urine.[69,70] Decreased expression or saturation of megalin receptor in diabetic PT likely underlies this process. This is further supported by a recent study, reporting a marked increase in urinary renin when the megalin receptor is antagonized.[54] In DKD, therefore, the source of urinary renin is increased filtered renin, through an abnormal glomerular barrier, which cannot efficiently be reabsorbed by the proximal tubule.

In conclusion, in humans with type 1 diabetes mellitus and DKD, as well as in mice with streptozotocin-induced diabetes mellitus, urinary renin is increased. Our data show that impaired proximal tubular reabsorption of filtered renin contributes to the increase of urinary renin in DKD likely due to decreased expression of megalin receptor. Studies in the renin-reporter mice made diabetic by streptozotocin, moreover, provide no evidence for migration of cells of the renin lineage to CDs that could be responsible for renin secretion and thus increased urinary renin in diabetes mellitus.

Perspectives

Renin is the key enzyme within the RAS that generates Ang I by hydrolyzing its unique substrate, angiotensinogen. As such, activation of the RAS is essentially dependent on circulating levels of renin produced by the JGA. It has been suggested, however, that the kidney CDs may also contribute to renin formation. In patients with DKD, PRA is usually decreased, but there is limited information on urinary renin and its origin. In the present study, we studied urinary renin from patients with type 1 diabetes mellitus and DKD and from mice with streptozotocin-induced diabetes mellitus and showed that urinary renin is elevated. By immunohistochemistry studies in mice with streptozotocin-induced diabetes mellitus, it was found that renin staining was reduced in the JGA but increased in the CDs. Using a renin-reporter mouse model made diabetic by streptozotocin administration, it is shown that this increase in urinary renin cannot be attributed to production from cells of the renin lineage migrating to the CD in a chronic hyperglycemic environment. The findings obtained by infusing L-lysine to block proximal tubular reabsorption moreover suggest that much of the urinary renin originates from filtered renin that cannot be reclaimed by the proximal tubule. Reduced megalin expression in the setting of DKD may account for reduced proximal reabsorption and therefore increase urinary renin. Future studies in patients with DKD should examine if renin staining in the JGA is decreased as demonstrated here in diabetic mice.

Sources of Funding

This work was supported by National Institute of Diabetes and Digestive Kidney Diseases grants R01DK080089 and R01DK104785 as well as by a gift to Northwestern University by the Joseph and Bessie Feinberg Foundation (D. Batlle). J. Tang had support from the Biomedical Education Program (BMEP).

Disclosures

None.

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Novelty and Significance

What Is New?

- Urinary renin is increased in a cohort of patients with type 1 diabetes mellitus and chronic kidney disease.
- Distribution of renin lineage cells was investigated for the first time in a renin-reporter mouse model made diabetic.

What Is Relevant?

- This study provides insight on the origin of urinary renin and its relevance in the setting of diabetic kidney disease.
- This is important because the activation of the renin-angiotensin system within the kidney causes sodium retention, hypertension, and progression of chronic kidney disease.

Summary

In humans with diabetic kidney disease and mice with diabetes mellitus induced by streptozotocin, urinary renin is increased. The source of this increase is largely attributable to increased filtration and impaired proximal tubular reabsorption. Filtered renin rather than local production of renin from migration of cells of the renin lineage to the renal collecting ducts in a hyperglycemic environment accounts for the bulk of urinary renin in diabetic kidney disease.