Renin Expression in Renal Ablation

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To determine whether expression of the renin-angiotensin system (RAS) is influenced by the degree of renal ablation, male Sprague-Dawley rats underwent uninephrectomy, 1/3 nephrectomy, or sham operation. Renin and angiotensinogen messenger RNA (mRNA) were not different among the three groups 2 weeks after surgery. The time course of expression of renin mRNA after 1/3 nephrectomy showed no difference versus controls at 2 and 4 weeks and a decrease at 6 weeks after surgical ablation. Because nephrons adjacent to the infarcted area in the 1/3 nephrectomy may be hypoperfused and a source of increased renin synthesis, intrarenal distribution of tissue renin content, renin mRNA, and immunostainable renin were examined in separate groups of rats subjected to 1/3 nephrectomy. The kidney was divided into two pieces, one containing the scar and scar-adjacent tissue and the other portion the tissue distant from the scar. Tissue renin content, renin mRNA, and immunostainable renin were significantly greater in the scar-adjacent tissue compared with the nonscar tissue. Immunoreactive renin was seen in the juxtaglomerular apparatuses as well as in vascular elements proximal to the juxtaglomerular apparatus and within mesangial cells of some glomeruli of the scar-adjacent tissue. In conclusion, immunostainable renin, tissue renin content, and renin mRNA were increased in scar-adjacent tissue after 1/3 nephrectomy. We speculate that this unique scar-associated redistribution of renin may play a pathophysiological role in the progression of renal disease. (Hypertension 1992;20:483-490)

Key Words • renin • angiotensin II • angiotensinogen • kidney • nephrectomy • ischemia • juxtaglomerular apparatus

Extensive evidence has shown that systemic and glomerular hypertension accelerate the progression of diverse forms of renal disease.1-4 The renin-angiotensin system (RAS) has an important role in the physiological regulation of the renal microcirculation.5,6 Angiotensin II (Ang II) exerts a vasoconstrictive effect, predominantly on the efferent arteriole, which would result in an increase in glomerular pressure.7,8 Evidence supporting a role for the RAS in the progression of renal disease includes the hemodynamic, permselective, and histological improvements seen in several models of renal disease with angiotensin converting enzyme inhibitors (CEI)1-2 or Ang II antagonists.9,10 In humans CEI have an antiproteinuric effect and slow the progression of established renal disease, effects that may be greater than those obtained with other antihypertensive drug therapies.11 Despite these clinical effects of the RAS, plasma renin activity (PRA) is generally low in chronic renal disease.2,12 The purpose of the present study was to examine the expression of the local intrarenal renin-angiotensin system after different degrees of renal ablation by measuring renal renin and angiotensinogen messenger RNAs (mRNAs). In a separate series of experiments we explored the intrarenal distribution of immunostainable renin, tissue renin content (TRC), and renin mRNA in the subtotal nephrectomy model.

Methods

Experimental Design

Experiment 1. The first experiment examined the effect of the degree of renal ablation on renal renin and angiotensinogen mRNA. Seventeen male Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) weighing 250-275 g were subjected to either right uninephrectomy (UNX) (n=5), right nephrectomy and ligation of the posterior branch of the left renal artery (1/3 NX) (n=6), or sham operation (n=6). These rats were maintained on a semisynthetic 20% protein diet (diet 170597, Teklad, Madison, Wis.). Two weeks after the operation, the left kidney was removed, snap-frozen in liquid nitrogen, and stored at -70°C for future RNA extraction.

Experiment 2. In a second experiment we explored the time course of expression of renin mRNA after 1/3 NX. Eighteen male Sprague-Dawley rats weighing 250-275 g underwent 1/3 NX (n=9) or sham operation (n=9). Rats were maintained on the same dietary regimen as described in the previous experiment. At different times after the surgery (2 weeks, 4 weeks, and 6 weeks) rats were killed (n=3 each time) and the left kidneys removed. All the specimens were snap-frozen in liquid nitrogen and stored at -70°C.

Systolic pressure was measured in a separate group of 11 rats subjected to either 1/3 NX (n=6) or sham...
operation (n=5). Blood pressure measurements were performed by the tail-cuff method before and at weekly intervals for the 6 weeks after the surgical procedure.

Experiment 3. In a third group of experiments we examined the intrarenal distribution of renin mRNA, TRC, and immunostainable renin after 2 weeks on NX. Eight Sprague-Dawley rats weighing 250–275 g were subjected to 1½ NX. These rats were also maintained for 2 weeks on the same semisynthetic diet, at which time the left kidney was removed and divided longitudinally into two pieces, one having the scar plus the scar-adjacent tissue (posterior half) and the other with the renal tissue “distant from the scar” (anterior half) (See Figure 1). The two pieces were snap-frozen in liquid nitrogen and stored at −70°C. Total RNA in each piece was extracted separately.

For TRC measurement, six 1½ NX male Sprague-Dawley rats were killed 2 weeks after partial renal ablation; the remaining kidney was removed and divided into scar-adjacent and non-scar pieces as described above. TRC was determined separately in each piece.

For light microscopy and renin immunoperoxidase studies, four male Sprague-Dawley rats weighing 250–275 g were subjected to either sham operation (n=2) or 1½ NX (n=2). Rats were maintained for 2 weeks on the same diet as described above, at which time they were anesthetized with methohexital, and the left kidney was perfusion-fixed with 10% formaldehyde. The kidney was harvested and sagittal sections obtained and processed as described below.

RNA Extraction and Northern Blot Hybridization

Total RNA from the kidney fragments was isolated using a modification of the guanidinium-isothiocyanate/cesium chloride method. The RNA was dissolved in sterile water and RNA concentrations determined by absorbance readings at 260 nm. Aliquots (20 µg) of total RNA were separated by electrophoresis in a 1% agarose gel containing 20 mM MOPS, 1 mM EDTA, 5 mM sodium acetate, pH 7.0, and 2.2 M formaldehyde, and were transferred to nylon membranes (Duralon UV, Stratagene, La Jolla, Calif.). In each gel, equivalent loading of RNA, absence of degradation, and the position of the 28S and 18S ribosomal RNA was determined by ethidium bromide staining. RNA was fixed to the nylon membrane by ultraviolet crosslinking (Stratalinker, Stratagene). The membranes were prehybridized at 60°C for 4 hours in a buffer containing 5× standard saline citrate (SSC), 5× Denhardt’s reagent, 50 mM tris-hydrochloride, pH 7.5, 0.1% sodium pyrophosphate, 0.2% sodium dodecyl sulfate (SDS), 200 µg/ml sonicated, denatured salmon testes DNA, and 100 µg/ml yeast transfer RNA (tRNA). The membranes were then hybridized at 42°C with random oligomer–primer–labeled complementary DNA (cDNA) probes (see below) for 16–18 hours in a buffer containing 50% deionized formamide, 5× SSC, 1× Denhardt's reagent, 50 mM tris-hydrochloride, pH 7.5, 0.1% sodium pyrophosphate, 1% SDS, 100 µg/ml salmon testes DNA, and 100 µg/ml yeast tRNA. The membranes were washed for 45 minutes in 2× SSC and 0.1% SDS twice at room temperature and once at 60°C, and were then washed in 0.2× SSC and 0.1% SDS at 60°C for 45 minutes. Autoradiographs (Kodak XAR-5 film) were obtained and quantified by computer-assisted videodensitometry. A standard curve was created to convert digitized gray values to true optical densities. This was accomplished by digitizing a calibrated step sensitivity guide (Stouffer Graphic Arts Equipment Co. Inc., South Bend, Ind.), calculating the mean grey value for each step, and creating a conversion table for all possible grey values (0 to 255).

Solution Hybridization

The presence of a single band for renin mRNA (1,600 bp) on hybridization of the nylon filters, permitted quantitation of renin mRNA by solution hybridization based on a modification of the technique of Durnam and Palmiter. Total kidney RNA (25–60 µg) was hybridized with approximately 20,000 cpm of a sulfur-35–labeled renin complementary RNA (cRNA) at 80°C in 20 µl of 0.6 M NaCl, 10 mM Tris-HCl, pH 6.5, 5 mM EDTA, 2.5% ethanol, and 0.1% SDS. The samples were digested with 1 ml RNase solution (RNase A [25 µg/ml], RNase T1 [250 units/ml], 0.3 M NaCl, 10 mM Tris-HCl, pH 7.5, 2 mM EDTA, and 75 µg salmon testes DNA) to digest unhybridized probe, precipitated with trichloroacetic acid, and quantified by liquid scintillation counting. A standard curve was constructed using mRNA transcribed by SP6 RNA polymerase after linearization of the renin plasmid by HindIII, (2–10,000 pg). The hybridized counts per minute of the unknown samples were fitted to the curve using a nonlinear sigmoid function as described by Rodbard.

Complementary DNAs and Preparation of the Complementary DNA and Complementary RNA Probes

The following cDNA probes were used: rat renin (pRen 44.ceb) and rat angiotensinogen (pRANG 6). cDNAs were a gift of K.R. Lynch, and mouse α-actin
cDNA (pAM91). The actin cDNA includes the protein-coding region and therefore it would recognize any actin mRNA regardless of isoform. For preparation of the cDNA probes we used the method of random oligomer-primer labeling (Promega, Madison, Wis.) with [γ-32P]ATP (6,000 Ci/mmol; New England Nuclear, Du Pont Co., Boston, Mass.). The specific activity of the probe was 1–2×10^6 cpm/μg DNA. For preparation of the cRNA probe, the renin plasmid was first linearized with the restriction enzyme BamHI. Two hundred and fifty microCuries of 32P-UTP (1,320 Ci/mmol; New England Nuclear) was evaporated to dryness in a microfuge tube and the following added: 1 μg linearized cDNA, 2.5 μl water, 2.0 μl 5× buffer (200 mM Tris-HCl, pH 7.5, 30 mM MgCl₂, 10 mM spermidine), 1.0 μl T7 RNA polymerase. The mixture was incubated for 60 minutes at 37°C. The reaction mixture was DNase-treated and unincorporated nucleotides removed by chromatography on a Sephadex G50 column.

Biochemical Methods

For determination of TRC, the kidney aliquots were kept at −70°C until their renin content was measured. The kidney was homogenized in a buffer containing 2.6 mM EDTA, 1.6 mM dimercaprol, 3.4 mM 8-OH quinoline sulfate, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), and 5 mM ammonium acetate, spun at 5,000 rpm, and the supernatant was removed and frozen and thawed four times. TRC was determined on an aliquot of supernatant after dilution to 1:1,000 by the quantitation of generated angiotensin I (Rianen Assay System, Du Pont Co., Billerica, Mass.). Two hundred microliters of the sample was incubated for 1 hour with 100 μl plasma obtained from nephrectomized male rats, 50 μl 4% EDTA, 10 μl 1.7% dimercaprol, 10 μl 6.6% 8-OH quinoline sulfate, and 630 μl 2 M maleate buffer (pH 6.0). TRC per milligram protein was determined by dividing TRC by the protein concentration of the aliquot of kidney assayed. Serum creatinine was measured with creatinine autoanalyzer (Beckman Instruments, Brea, Calif.).

Immunohistochemistry

A polyclonal rabbit anti-rat renin antiserum, which has been previously reported and characterized (provided by Dr. T. Inagami), was used for the immunoperoxidase studies. Sections from formalin-fixed, paraffin-embedded tissue were deparaffinized in Americlear (American Scientific Products, Minneapolis, Minn.) and rehydrated in graded ethanol and then buffer. Endogenous peroxidase was blocked with 0.8% hydrogen peroxide in absolute methanol. The sections were incubated with 2% sheep serum to block nonspecific binding and then stained with the peroxidase-antiperoxidase procedure as previously described. After overnight incubation at 4°C with the primary antibody (rabbit anti-rat renin polyclonal antibody), the sections were incubated with sheep anti-rabbit antiserum (diluted 1:80) (Antibodies Inc., Davies, Calif.) and with rabbit antiperoxidase-antiperoxidase (diluted 1:300) (Sternberger Meyer Immunochemicals Inc., Jarrettsville, Md.). The reaction was demonstrated with 3–3 ′ diaminobenzidine tetrahydrochloride (0.25 mg/ml) (Sigma Chemical Co., St. Louis, Mo.) and 0.03% peroxide; the sections were counterstained with Harris' hematoxylin. Positive controls were represented by sections from tissue known to contain the antigen of interest; negative controls consisted of sections in which normal rabbit serum was substituted for primary antiserum.

Statistical Analysis

Statistical significance is defined as p<0.05, and the results are presented as mean±SEM. The significance of the differences was analyzed by either the Student's t test of unpaired data or one-way analysis of variance when three group comparisons were made. Student-Newman-Keuls test was used for intergroup comparisons.

Results

Experiment 1

General parameters. The mean body weights of the sham, UNX, and 1½ NX groups were not different at either the start or end of the study (Table 1). The wet weight of the left kidney was greater in the UNX and 1½ NX groups compared with the sham-operated group (Table 1). Similarly, the kidney weight/body weight ratio was also greater in the UNX and 1½ NX groups compared with the sham group (Table 1). Serum creatinine at the end of the 2-week study period was significantly different in the three groups; the highest values, as expected, occurred in the 1½ NX group (Table 1). Food intake was quantitated on 5 consecutive days (day 6 to day 10 of the study period) and was not different among the three groups (sham, 26.3±1.3 versus UNX, 28.0±2.1 versus 1½ NX, 28.3±1.8 g; p=NS).

Northern and solution hybridization. Analysis of Northern blots confirmed a single band for renal renin mRNA (1,600 bp), angiotensinogen mRNA (1,800 bp), and a predominant band for actin (2,200 bp) mRNA. Figure 2A shows an autoradiograph of the Northern blot of RNA extracted from the left kidney of six sham-operated, five UNX, and six 1½ NX rats, hybridized with a renin cDNA probe. Quantitation by computer-assisted densitometry demonstrated that the relative renin mRNA level was not different among the three groups (sham, 306±36 versus UNX, 282±43 versus 1½ Nx, 310±82 optical density [O.D.] units; p=NS). The same Northern blot was hybridized with an angiotensinogen cDNA probe. Quantification by densitometry showed that the mRNA level for angiotensinogen was also not different for the three groups (sham, 58±4 versus UNX, 52±5 versus 1½ Nx, 52±4
A. Renin

B. Angiotensinogen

C. Actin

FIGURE 2. Autoradiographs show Northern blot of total kidney RNA (20 μg) extracted from six sham-operated rats, five rats with uninephrectomy (UNX) and six rats with 1/3 nephrectomy (1/3 NX) hybridized with renin (panel A), angiotensinogen (panel B), and α-actin (panel C).

O.D. units; p=NS) (Figure 2B). The Northern blot was also hybridized with an actin probe and quantified by videodensitometry with the following results: sham, 386±42 versus UNX, 234±15 versus 1/3 NX, 320±18 O.D. units; p<0.05 UNX versus sham (Figure 2C). Renin mRNA was also quantified by solution hybridization and was not significantly different among the three groups (sham, 2.53±0.7 versus UNX, 2.36±0.23 versus 1/3 Nx, 2.63±0.48 pg renin mRNA per μg total RNA; p=NS).

Experiment 2

Figure 3 shows an autoradiograph of the Northern blot of RNA extracted from the left kidney of sham-operated and 1/3 NX rats killed at different time points (2, 4, and 6 weeks) and hybridized with a renin cDNA probe. At each time point, three sham and three 1/3 NX rats were included in the analysis. There was no difference in renin mRNA between the sham and 1/3 NX groups at 2 and 4 weeks, whereas renin mRNA was significantly reduced in the 1/3 NX group at 6 weeks (2 weeks: sham, 300±44 versus 1/3 NX, 373±36; 4 weeks: sham, 336±59 versus 1/3 NX, 275±54; 6 weeks: sham, 235±30 versus 1/3 NX, 107±14 O.D. units) (see Figure 3).

FIGURE 3. Autoradiograph shows Northern blot of RNA extracted from the left kidney of sham-operated and 1/3 nephrectomy (1/3 NX) rats killed at different time points (2, 4, and 6 weeks), and hybridized with a renin complementary DNA probe.

The 1/3 NX group had significantly higher systolic blood pressure when measured at 1 week after renal ablation, and this elevation persisted throughout the 6 weeks of the study (Figure 4).

Experiment 3

Renin mRNA. A Northern blot hybridized with a renin cDNA probe of total kidney RNA extracted from the two portions of the kidneys of two rats demonstrated an increased level of renin mRNA in the scar-adjacent tissue as compared with the non-scar tissue (scar-adjacent, 301±8 versus non-scar, 152±37 O.D. units). Duplicate Northern blots, hybridized with actin (scar-adjacent, 1,305±329 versus non-scar, 1,579±191 O.D. units) and angiotensinogen (scar-adjacent, 52.3±7 versus non-scar, 52.4±9 O.D. units) probes, showed no difference in the level of the message between scar-adjacent and non-scar kidney fragments, demonstrating the specificity of the increase in renin mRNA in the scar-adjacent tissue. When renin mRNA was quantified by solution hybridization (n=8 of each), it was significantly higher in the tissue adjacent to the scar compared with the tissue distant from the scar (4.22±0.53 versus 2.09±0.23 pg renin mRNA per μg total RNA; p<0.01) (Figure 5A), thus confirming the results of the Northern hybridization.

Tissue renin content. TRC was significantly greater in the scar-adjacent compared with the non-scar piece (scar-adjacent, 3.40±0.59 versus non-scar, 0.43±0.11 μg angiotensin I·mg protein⁻¹·hr⁻¹; p<0.01) (Figure 5B). Thus, the increase in both renin mRNA and tissue renin content in the scar-adjacent tissue provide evidence for a redistribution of renin after 1/3 NX.

Histopathology and immunohistochemistry. The sham group demonstrated no histological abnormalities on light microscopy. Two weeks after 1/3 NX three distinct zones were identified in the remnant kidney (illustrated in Figure 6A) which is the negative serum control for.
the immunoperoxidase studies. The scar zone was characterized by loss of the normal renal architecture with replacement by fibrosis and areas of calcification. A distinct rim of tissue adjacent to the scar was easily identifiable. Tubular atrophy was present in this scar-adjacent tissue. The tissue distant from the scar showed no morphological abnormalities. The immunohistochemical study of the sham group demonstrated the presence of immunostainable renin in the juxtaglomerular apparatuses of those glomeruli whose section allowed examination of this anatomical region of the glomerulus (Figure 6B). In the 1½ NX group the distribution of immunoreactive renin was heterogeneous and related to the anatomical zones previously described: the renal tissue distant to the scar had a decreased number of immunoreactive juxtaglomerular cells, and in those cells that were positive, the intensity of the reaction was clearly diminished in relation to the sham group (Figure 6C). The scar-adjacent zone showed an increased number of juxtaglomerular cells reactive for renin as well as an increase in the intensity of staining of each juxtaglomerular region (Figure 6D). In addition, there was evidence of renin immunoreactivity in vascular elements proximal to the juxtaglomerular apparatus (Figure 6E) and in the glomerular tuft within mesangial cells (Figure 6F). The scarred area was negative for renin immunostaining. The results of the immunohistochemical studies are based on the histological study of only two rats in each group. However, the demonstration of a redistribution of renin to the scar-adjacent tissue is consistent with the renin mRNA and TRC data.

**Discussion**

In the current study, we demonstrate that the fractional renin mRNA level was not significantly different between the UNX, 1½ NX, or sham groups at 2 and 4 weeks after the surgical procedure, but was reduced in the 1½ NX group at 6 weeks. Systolic blood pressure was significantly higher in the 1½ NX group 1 week after ablation and remained elevated throughout the study. We also demonstrated a pattern of redistribution specific for renin in the 1½ NX, as evidenced by the increase in TRC and renin mRNA in scar-adjacent tissue, with no change in actin or angiotensigen mRNAs. This pattern of redistribution was further supported by the increased immunoreactive renin in the scar-adjacent tissue. In addition, the scar-adjacent area of the 1½ NX kidney demonstrated the presence of renin immunostaining in vascular segments proximal to the juxtaglomerular apparatus as well as distally in the glomerular tuft within mesangial cells. In contrast, renin was suppressed in the tissue distant from the scar. Since renin is the rate-limiting step in the generally nonsubstrate-dependent reaction leading to Ang II formation, an increase in renin mRNA and TRC in the tissue adjacent to the scar suggests, but does not prove, that this is an area of increased Ang II generation.

Chronic renal failure is generally accompanied by a decreased PRA. On the other hand, progressive renal disease favorably responds to several manipulations likely to reduce levels of Ang II such as dietary protein restriction and angiotensin converting enzyme inhibition. This constitutes an apparent paradox. In recent years, evidence has accumulated to support the existence of a local intrarenal RAS, with the demonstration that all the components necessary for the synthesis of Ang II are present within the kidney. Therefore, the lack of an increase in PRA does not exclude Ang II from having important local effects in the kidney. The role of local intrarenal Ang II in the pathogenesis of glomerular injury may proceed through hemodynamic as well as nonhemodynamic pathways.

Nephrons adjacent to an infarcted area demonstrate morphological evidence of ischemia without infarction and our current study confirms this finding. Glomeruli in the border zone adjacent to a scar may be perfused by retrograde filling from the capillaries of the contiguous noninfarcted tissue. This finding raises the possibility that such hypoperfused nephrons are a source of increased renin synthesis. That activation of the RAS in the tissue bordering the scar after renal ablation by infarction may occur is suggested by the study of Meyer.
and Rennke, who compared the course of renal disease in rats with bilateral infarction of 40% of their renal mass to uninephrectomized rats. The group of rats with limited bilateral infarction exhibited more glomerulosclerosis, more severe proteinuria, and increased systemic and glomerular pressure compared with the rats that had undergone uninephrectomy. Additional indirect evidence suggesting activation of the RAS in the scar-adjacent tissue is the fact that when renal mass is removed surgically (polectomy) and not by infarction, less severe hypertension develops. Therefore, the increase of renin mRNA in the scar-adjacent tissue is consistent with the presence of local ischemia and stimulation of the local RAS. Based on the solution hybridization data, we estimate the level of renin mRNA in the scar-adjacent tissue was at least twice that of the normal serum control.
seen in the kidneys of sham-operated rats. Since renin mRNA in these assays is expressed as a fraction compared with total RNA, the amount of renin mRNA in the scar-adjacent tissue would, however, be dependent on the amount of "normal" tissue included in the sample. This local elevation in renin would likely generate increased Ang II, which could have effects on more distant nephrons, since Ang II and renin diffuse through the interstitium and conversion of angiotensin I to Ang II can occur at this level.31

The human kidney in advanced renal diseases as well as the remnant kidney in the rat demonstrates widespread corticomедullary fibrosis. This scarring process correlates closely with a deterioration of functional parameters, including hemodynamic as well as tubular transport functions.32 The mechanisms by which tubulointerstitial injury appears and is perpetuated, even in primary glomerular diseases, are not well understood. However, Diamond and Anderson33 showed that Ang II blockade by converting enzyme inhibitors lessens tubulointerstitial injury induced by puromycin aminonucleoside in rats. Global glomerulosclerosis is also a feature of progressive renal disease. Increased renin immunostaining has been shown in the area adjacent to some obsolescent glomeruli.34 Also, immunostaining of renin in tissue specimens of patients with renal infarcts secondary to thrombosis was increased in the ischemic areas as compared with nonischemic areas of the same kidney.35 These findings suggest that scar-related activation of the RAS is of potential pathophysiological importance in human renal disease.

We have previously described renin synthesis in glomeruli of remnant kidneys.36 The remnant glomerular tuft probably becomes a site of renin synthesis since immunostainable renin accumulates at this level and renin gene expression is significantly elevated in glomeruli from remnant kidneys 4 weeks after 1/3 nephrectomy as compared with that of control glomeruli.36 In the current study, distribution of renin mRNA was assessed by immunohistochemistry at an earlier timepoint (2 weeks) and after a less severe degree of renal ablation (1/3 NX). Despite these differences in design, immunostainable renin was found within mesangial cells of scar-adjacent glomeruli. A second pattern of redistribution that has not been previously shown for the remnant kidney is the presence of renin immunoreactivity in vascular elements proximal to the juxtaglomerular apparatus in the ischemic renal parenchyma bordering the scar.

Variations in the anatomical distribution of immunostainable renin have been demonstrated in several experiments and clinical conditions. For example, after converting enzyme inhibition and during the course of normal kidney maturation in the rat, renin synthesis and storage migrate to proximal sites in the vascular tree.37,38 Distal migration of renin synthesis and storage to the glomerular tuft have been noted in states of mineralocorticoid deficiency and salt depletion as well as the remnant kidney.36,39 A state in which mesangial cells are probably the renin-producing cell line. In addition, mesangial cells can synthesize renin in vitro.40 Human renal biopsies have also shown variations in the distribution of vascular immunostaining of renin in several pathological conditions. Proximal extension of renin-secreting cells into the vascular tree has been described in polyarteritis nodosa41 and in renal artery stenosis42; it was postulated that the focal ischemia present in these entities can be an activating factor for the RAS. A similar type of pattern of renin immunoreactivity has been described in renal biopsies of patients with end-stage renal disease and dialysis-resistant hypertension.43 In adult polycystic kidney disease renin-containing cells were present not only in juxtaglomerular apparatuses of surviving nephrons, but also in interlobular arteries, and in some cases renin was identified within glomerular tufts.45 The functional significance of these intrarenal shifts of the RAS is unclear; however, local domains of increased renin or Ang II production may act on neighboring nephrons or particular components of the nephron, e.g., mesangium.

In conclusion, increased renin mRNA, TRC, and renin immunoreactivity were demonstrated in the scar-adjacent tissue in the 1/3 NX model. This unique redistribution of renin supports the hypothesis of local hypoperfusion and subsequent stimulation of renin synthesis in the tissue surrounding the infarctive scar.

Acknowledgments
We thank David Chmieliewski and Stefan Kren for expert technical assistance, Kevin Lynch for the renin and angiotensinogen cDNAs, and Tadashi Inagami for the rabbit anti-rat renin polyclonal antibody. We are grateful to Merck Sharp & Dohme for sponsorship of Figure 6 to be produced in color.

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Renin expression in renal ablation.
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Hypertension. 1992;20:483-490
doi: 10.1161/01.HYP.20.4.483

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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