Renal Injury From Angiotensin II–Mediated Hypertension

Richard J. Johnson, Charles E. Alpers, Ashio Yoshimura, Donna Lombardi, Pam Pritzl, Jürgen Floege, and Stephen M. Schwartz

Angiotensin II (Ang II)–mediated hypertension induces vascular smooth muscle cell hypertrophy and hyperplasia in systemic blood vessels, but the effects of Ang II on the intrinsic cell populations within the kidney have been less well characterized. We infused Ang II for 14 days into rats by minipump at doses (200 ng/min) that resulted in moderate hypertension (mean systolic blood pressure 156-172 mm Hg). Small renal arterial vessels of Ang II-infused rats demonstrated focal injury with fibrinoid necrosis and medial hyperplasia, whereas the glomerular capillaries demonstrated only rare segmental hyalinosis. Proliferation of vascular smooth muscle cells was pronounced (fourfold to 20-fold increase in [3H]thymidine incorporation) as opposed to a minimal proliferation of glomerular cells in Ang II-infused rats. In contrast, the principal effect of Ang II in glomeruli was to increase the expression of α-smooth muscle actin by mesangial cells and desmin by visceral glomerular epithelial cells. Ang II–infused rats also developed focal tubulointerstitial injury, with tubular atrophy and dilation, cast formation, an interstitial monocytic infiltrate, and mild interstitial fibrosis with increased type IV collagen deposition. The injury was associated with a proliferation of distal tubule, collecting duct, and interstitial cells as determined by immunostaining for proliferating cell nuclear antigen, and was accompanied by an increase in platelet-derived growth factor B-chain messenger RNA in the area of interstitial injury as localized by in situ hybridization. Renal interstitial cells also underwent phenotypic modulation in which they expressed α-smooth muscle actin. Vehicle-infused control rats displayed no tubular injury, proliferation, or phenotypic modulation. Thus, Ang II in doses that cause moderate hypertension induces marked vascular, glomerular, and tubulointerstitial injury with cell proliferation, leukocyte recruitment, phenotypic modulation with the upregulation of proteins normally associated with smooth muscle cells, and interstitial fibrosis. (Hypertension 1992;19:464-474)

KEY WORDS • angiotensin II • actins • vascular smooth muscle • kidney tubules • renovascular hypertension

Chronic angiotensin II (Ang II) infusion results in persistent hypertension that appears to involve several mechanisms, including a direct vasoconstrictive action of Ang II, increased production of aldosterone, increased sympathetic tone, and sodium retention.1,2 Furthermore, studies in rats have demonstrated that sustained hypertension may develop several weeks to months after a short series of daily infusions of Ang II have been administered.3 The mechanisms for the sustained hypertension are of intense interest. One potential mechanism by which Ang II may contribute to the sustained elevation of blood pressure is by direct or indirect effects of Ang II that directly mediate hypertension and that the hypertrophic response may be separated from effects of Ang II on the kidney. Over 40 years ago Wilson and Byrom4,5 demonstrated that Ang II–mediated hypertension, induced by clamping one renal artery in rats (i.e., the two-kidney, one clip Goldblatt model), leads to significant glomerular, tubular, and vascular injury in the unclipped (i.e., unprotected) kidney. Once histological injury was induced in the hypertensive kidney, removal of the clamp from the clipped kidney was ineffective in restoring blood pressure to normal in the majority of cases.7

Numerous studies demonstrate that the principal effect of Ang II–mediated hypertension on the peripheral vasculature involves both a hypertrophic and proliferative response.8–10 Both cell culture8,9 and in vivo10 studies suggest that this hypertrophic response may be separated from effects of Ang II on the kidney that directly mediate hypertension. The ability of Ang II–mediated hypertension to induce vascular smooth muscle proliferation in vivo has also been demonstrated.11,12 The mitogenic effect of Ang II may be partially independent of its ability to mediate hypertension,13,14 although studies of the effects of Ang II on vascular...
smooth muscle cells in culture have provided conflicting evidence.8,15,16

Given the evidence that Ang II can induce vascular hypertrophy and proliferation in vivo, we examined whether Ang II-mediated hypertension induces cell proliferation in the kidney, with emphasis on each of the major renal cell populations (glomerular, vascular, tubular, and interstitial). We have also examined whether upregulated expression of contractile proteins, particularly α-smooth muscle actin, occurs in each of these cell types. Our studies demonstrate distinct effects of Ang II-mediated hypertension to induce injury, cell proliferation, and phenotypic modulation of the various renal cell types. These studies may provide insights into the complexity of the renal response to Ang II, and the mechanisms underlying the sustained hypertension that occurs with Ang II.

Methods

Experimental Protocol

Studies were performed to determine if continuous administration of Ang II in the rat at a concentration that causes modest hypertension results in functional or morphological evidence of renal injury. Male Sprague-Dawley rats (Zivic Miller, Allison Park, Pa.) (400–550 g) (n=6) received continuous Ang II infusion (200 ng/min) via subcutaneous osmotic minipumps (Alzet model 2002, Alza Corp., Palo Alto, Calif.) containing Ang II (Sigma Chemical Co., St. Louis, Mo.) dissolved in Ringer’s lactate.11 Control rats (n=6) had subcutaneous osmotic minipumps containing Ringer’s lactate. Both Ang II-infused and control rats also had a second osmotic minipump placed intraperitoneally that delivered [3H]thymidine at 4.5 μCi/hr for the 2-week study period. Rats in each group underwent renal biopsy by flank incision under ether anesthesia at 7 and 14 days and were then killed by sodium pentobarbital overdose.

Blood Pressure Measurement

Systolic arterial blood pressures were measured in conscious, restrained rats by tail-cuff plethysmography (Narco BioSystems, Austin, Tex.).11 Measurements were performed 2–4 times per week, beginning 3 days before the placement of the minipumps. The blood pressure value for each rat was calculated as the average of three separate measurements at each session.

Histology

Methyl Carnoy’s fixed tissue was paraffin embedded, processed, and 4-μm sections stained with Gomori’s trichrome or with the periodic acid/Schiff (PAS) reagent with hematoxylin counterstain.17 Additional 4-μm sections were immunostained using an indirect avidin-biotin immunoperoxidase method17 with specific monoclonal antibodies directed to the following antigens: human renin, with F37.2D12 (kind gift of M. Laprade, Sanofi Recherche, Montpellier, France);18 the proliferating cell nuclear antigen (PCNA)/cyclin, with 19A2 (Coulter Corp., Hialeah, Fla.);19 OX-22 (Accurate Chemical and Scientific Corp., Westbury, N.Y.); α-smooth muscle actin with anti-asma-1 (kind gift of G. Gabbiani, Geneva, Switzerland);20 γ-smooth muscle actin with B4 (kind gift of J. Lessard, Cincinnati, Ohio);22 and desmin with D33 (Dako Corp., Santa Barbara, Calif.). Type IV collagen was detected by immunoperoxidase using a rabbit anti-mouse type IV collagen antibody (Collaborative Research, Bedford, Mass.). Double immunolabeling of the same tissue sections was also performed with the anti-asma-1 and 19A2 antibodies using an immunoperoxidase method as previously described.17 Negative controls for the immunostaining consisted of replacing each of the primary antibodies with an equivalent concentration of irrelevant monoclonal or rabbit polyclonal antibody.

Autoradiography

The incorporation of [3H]thymidine into newly synthesized DNA was detected by autoradiography. Tissue sections (4–μm) were dipped in Kodak NTB-2 emulsion (diluted 1:1 with distilled H2O) and stored for 3 weeks at 4°C. The sections were then developed, fixed, and counterstained with hematoxylin, as previously described.11 The presence of three or more grains per nucleus was considered positive.

In Situ Hybridization

The detection of platelet-derived growth factor (PDGF) B-chain messenger RNA (mRNA) in formalin-fixed tissue from control rats (n=4) and Ang II-infused rats (n=4) was performed using in situ hybridization with a specific antisense complementary RNA (cRNA) probe as detailed previously.24 Both sense and anti-sense digoxigenin-labeled cRNA probes were constructed from murine PDGF B-chain genomic DNA (a 326-bp fragment containing 135 bp Exon 6) (gift of Charles Stiles, Boston, Mass.) as previously described.24 Hybridization was performed overnight at 55°C using 300-ng/ml cRNA probe. Detection was accomplished with an anti-digoxigenin antibody conjugated to alkaline phosphatase (Genius Nonradioactive Nucleic Acid detection kit, Boehringer-Mannheim, Mannheim, FRG), followed by colorimetric reaction.24 Negative controls included 1) hybridization with a sense probe to matched serial sections; 2) hybridization with the anti-sense probe following incubation of the tissue with 20 μg/ml RNAase A (Sigma); and 3) absence of color reaction when either the probe, detecting antibody, or color solution were deleted.24

Quantitation of Morphological Data

The mean number of proliferating cells (PCNA+), macrophages (ED-1+ cells), and neutrophils (RP-3+ cells) per glomerular cross-section in each biopsy was determined by sequentially examining 20 glomeruli with more than 20 capillary loops per glomerular cross-section. The mean number of proliferating cells (PCNA+), macrophages (ED-1+ cells), and neutrophils (RP-3+ cells) in the tubulointerstitium of each biopsy was calculated by enumerating the number of positive cells in 50 sequentially selected 1-mm² grids (chosen to exclude large blood vessels or glomeruli) at 400 magnification. The number of proliferating cells in blood vessels was determined by autoradiography by
calculation the labeling index (% positive cells) of the endothelial cell, smooth muscle cell, and adventitial cell layers. The degree of glomerular staining for α-smooth muscle actin was graded from 0–4+ as previously described.23 A semiquantitative estimate of renal renin content was obtained by determining the percent of glomerular cross-sections (using at least 30 glomeruli per biopsy) with juxtaglomerular staining for renin with the monoclonal anti-renin antibody.

Localization of Aminopeptidase A (Angiotensinase A)

Kidney tissue was snap frozen in isopentane, and 4-μm sections were incubated with L-glutamic acid α-(4-methoxy-β-naphthylamide) (Sigma) (0.25 mg/ml) as substrate using Fast Blue B (Serva, Heidelberg, FRG) (1 mg/ml in 0.1 M phosphate buffer, pH 6.8, and 1.5 mM CuCl₂) as a coupling salt.36

Immunoelectron Microscopy

Additional rats were infused with Ang II (n=3) or vehicle (n=3), and the right kidney was perfused with periodate-lysine-paraformaldehyde (PLP) at 7 days and processed for immunoelectron microscopy (immuno-EM) as previously described.17 Thin sections were stained with HHF-35, an antibody to the muscle actin isoforms27 and detected with goat anti-mouse immunoglobulin G (IgG) conjugated to gold (10-nm particles) (Sigma). Controls consisted of replacing the HHF-35 with an irrelevant monoclonal antibody. Grids were processed for immunoelectron microscopy (immuno-EM) as previously described.17 Thin sections were stained with HHF-35, an antibody to the muscle actin isoforms27 and detected with goat anti-mouse immunoglobulin G (IgG) conjugated to gold (10-nm particles) (Sigma). Controls consisted of replacing the HHF-35 with an irrelevant monoclonal antibody. Grids were treated with uranyl acetate and examined with a Phillips 410 electron microscope.

Blood urea nitrogen (BUN) was measured by autoanalyzer.

Statistical Analysis

Values are expressed as mean±SD. Comparisons between groups were made using the Student's t test. A value of p<0.05 was considered significant.

Results

Hemodynamics

Blood pressures in the control and Ang II rats were in the normotensive range before administration of Ang II, although blood pressures were lower in the rats that were to receive Ang II (Table 1). After placement of subcutaneous osmotic minipumps that delivered Ang II at a constant rate of 200 ng/min, mild-to-moderate hypertension developed in the rats (Table 1). Blood pressures in Ang II-infused rats remained in the normal range through day 3 (blood pressure 137±10 mm Hg, day 3), but the rats then became progressively hypertensive (Table 1). One Ang II-infused rat had minimal elevations of blood pressure during the first week (range 95–145 mm Hg). Mean systolic blood pressures in vehicle-infused rats remained less than 140 mm Hg during the study (Table 1).

Effect of Angiotensin II Infusion on Renal Renin Content

Light microscopy of PAS-stained tissue suggested that there was a reduction in the size of the juxtaglomerular apparatus in Ang II–infused rats. This was supported by studies in which kidney tissue was stained for renin with a monoclonal antibody to human renin. In control rats, over 40% of glomerular cross-sections stained for renin in the juxtaglomerular regions, whereas this was significantly reduced in Ang II–infused rats (32±8% versus 48±4% at day 7, p<0.005; 27±7% versus 40±3% at day 14, in Ang II–infused versus control rats, p<0.005).

Glomerular Changes

By routine light microscopy (i.e., PAS-stained tissue sections), the majority (more than 90%) of glomeruli appeared normal in Ang II–infused rats. Occasional glomeruli in Ang II–infused rats showed features of segmental hyalinosis, whereas glomeruli of vehicle-infused control rats were always normal.

To determine if Ang II infusion induces glomerular cell proliferation, tissue sections were immunostained for the PCNA, a nuclear protein that is expressed from late G1 through the M phase of the cell cycle.28 A small increase in proliferating cells (PCNA+ cells) could be documented in glomeruli of Ang II–infused rats that was significant at day 14 (Table 2). This mild increase in glomerular cell proliferation in Ang II–infused rats was also verified by measuring the number of [3H]TdT-positive glomerular cells by autoradiography (0.5±0.2 versus 0.16±0.05 [3H]TdT+ cells/glomerular cross-section in Ang II versus control rats, day 14, n=4 per group, p<0.01).

Leukocyte infiltration in glomeruli of Ang II–infused rats, as documented by enumerating neutrophils and monocyte-macrophages with cell-type specific antibodies (i.e., RP-3 and ED-1, respectively), was minimal (Table 2).

The administration of Ang II resulted in a dramatic phenotypic modulation with the induction of smooth muscle cell–like proteins by glomerular cells. Whereas most control glomeruli were either negative or only had
FIGURE 1. Photomicrographs show glomerulus from a control rat that has been immunostained for α-smooth muscle actin (panel a) showing positive afferent and efferent arterioles, but the mesangium is negative. In contrast, the glomerulus from an angiotensin II (Ang II)-infused rat at 7 days (panel b) shows dramatic upregulation of this actin isoform. In the control rat glomerulus, desmin is localized by immunostaining to the mesangium with variable expression by the visceral glomerular epithelial cells (panel c). Glomeruli from Ang II-infused rats show a marked increase in desmin content, particularly of the visceral glomerular epithelial cells (arrows) (panel d). Magnification ×400.

trace staining for α-smooth muscle actin, glomeruli from Ang II-infused rats had marked staining of cells in mesangial regions (1.8±0.2 versus 0.5±0.2 in Ang II versus vehicle-infused rats, scale 0–4+, at day 7, p<0.001; 1.5±0.4 versus 0.3±0.2 at day 14, p<0.001) (Figure 1). The mesangial pattern of staining suggested that most of the α-smooth muscle actin was expressed by the smooth muscle cell-like mesangial cell. In contrast to α-smooth muscle actin, γ-smooth muscle actin could not be demonstrated in glomeruli of either Ang II-infused or control rats, although light immunostaining of the blood vessels was documented in both groups.

Ang II-mediated hypertension was also associated with increased glomerular expression of desmin, a muscle-associated intermediate filament protein. In vehicle-infused rats, desmin localized to the mesangium with variable expression by visceral glomerular epithelial cells, as has been reported for normal rats17 (Figure 1C). In contrast, Ang II-infused rats had a marked increase in desmin content in glomeruli that was localized primarily to the visceral epithelial cells (i.e., podocytes) (Figure 1D).

To determine if Ang II modulated the production of Ang II–degrading enzymes by glomerular cells, we stained renal cortical tissue for aminopeptidase A using a histochemical enzymatic assay. Aminopeptidase A (previously referred to as angiotensinase A) is an enzyme that inactivates Ang II by removing the N-terminal Asp1 of Ang II.29 In control animals, positive staining of aminopeptidase was present in proximal tubules and in the glomerular capillary wall (Figure 2A). In Ang II-infused rats, the glomerular staining was qualitatively increased in glomeruli at both days 7 and 14 (Figure 2B). Tubular staining was variable between individual animals, and no obvious differences in tubular staining were noted in Ang II-infused versus control rats.

Vascular Injury and Proliferation

At 7 days, one of six Ang II-infused rats demonstrated focal areas of fibrinoid necrosis by light microscopy (PAS stain) involving the afferent arteriole and other small arteries and arterioles. By 14 days, four of six Ang II–infused rats showed an increase in cellularity in small arterial vessels, occasionally accompanied by foci of fibrinoid necrosis. All four rats had been hypertensive throughout the period of Ang II infusion. Vehicle-infused rats had no vascular abnormalities at either time studied.

To determine if the vascular injury involved cell proliferation, tissue was immunostained for PCNA. A marked proliferation of smooth muscle cells in the small arterial blood vessels and arterioles was demonstrated in Ang II–infused rats, as compared with controls at both days 7 and 14 (Figures 3A and 3B). At day 14, 4.74±2.0% of smooth muscle cells in small arterial vessels were PCNA positive, as compared with 1.45±0.9% of vascular smooth muscle cells in control
Interstitial Changes

There was no difference in the number of lymphocytes (i.e., OX-22 positive cells) present in the interstitium (0.6 ± 0.3 versus 0.7 ± 0.2 OX-22+ cells/mm² at day 7; 0.2 ± 0.2 versus 0.1 ± 0.1 RP-3+ cells/mm² at day 7; 0.1 ± 0.1 versus 0.2 ± 0.2 RP-3+ cells/mm² at day 14 in Ang II-infused versus control rats, p = NS). Focal areas of interstitial fibrosis were present in the renal cortex of Ang II-infused rats at 14 days (Figure 4). The fibrotic areas localized to areas with tubular atrophy and leukocyte infiltration and were associated with increased type IV collagen content by immunostaining (Figures 4E and 4F).

A dramatic increase in α-smooth muscle actin was demonstrated in the interstitium of the Ang II-infused rats (Figure 5). By immuno-EM, the cells expressing the muscle actin isoform could be shown to be spindle-shaped interstitial cells localized outside the peritubular capillary in the interstitial space (Figure 6). When the tissue sections were subjected to double immuno-labeling for both α-smooth muscle actin and for PCNA, the majority of α-smooth muscle actin positive cells were negative for PCNA: 10–15% of the α-smooth muscle actin positive interstitial cells were actively proliferating (i.e., PCNA+) at day 7, and 4–10% were PCNA+ at day 14.

Proliferation Is Associated With Increased Platelet-Derived Growth Factor B-chain Messenger RNA Expression

Tissue was examined at day 14 in both Ang II-infused rats (n = 4) and vehicle-infused rats (n = 4) for PDGF B-chain mRNA by in situ hybridization (Figure 7). Glomeruli of both Ang II-infused and control rats were usually negative, although occasionally one or two positive cells per glomerular cross-section could be identified. Both distal tubules and collecting ducts were focally positive for PDGF B-chain mRNA in control rats (Figure 7A). In contrast, in Ang II-infused rats qualitative increases in PDGF B-chain mRNA were present in the areas of tubular injury (Figures 7B and 7C). The increase in PDGF B-chain mRNA was diffusely localized to the expanded interstitial areas and tubules. The identification of individual cells in the interstitium expressing PDGF B-chain mRNA was often difficult but occasionally could be visualized (Figures 7B and 7C). Small arterioles and arteries also had occasional positive cells within the media in both Ang II-infused and control rats (Figures 7A and 7C). Tissue examined with a sense cRNA probe for the PDGF B-chain mRNA was negative (Figure 7D).

Renal Function

Ang II-infused rats had significantly worse renal function at 2 weeks compared with vehicle-infused rats,
as determined by BUN concentration (29.8 ± 9.8 versus 19.5 ± 1.5 mg/dl in Ang II-infused versus control rats, respectively, p < 0.05, n = 6 per group).

Discussion

In this study, the ability of Ang II–mediated hypertension to induce morphological injury to the kidney was studied, with an emphasis on whether injury was associated with proliferation or phenotypic modulation of the various renal cell populations. It has been previously reported that large (10–100 μg) bolus infusions of Ang II may induce severe glomerular and vascular injury,30 and long-term infusions of Ang II (i.e., 8 weeks) may result in glomerulosclerosis.31 Severe structural alterations also occur in the “unprotected” kidney in the two-kidney, one clip model of Ang II–mediated hypertension.6-7 In contrast, in the present study the infusion of Ang II resulted in only modest hypertension, and routine light microscopy revealed only focal areas of tubulointerstitial injury, often in association with injured blood vessels. Despite the apparent modest effects of Ang II–mediated hypertension on renal structure, the use of special immunohistochemical stains revealed a remarkable effect of Ang II to induce proliferation and phenotypic changes in the various renal cell populations.

Vascular Changes

The infusion of Ang II resulted in proliferative vascular injury with a marked increase in DNA synthesis in the smooth muscle cells of renal arterial blood vessels as documented by both immunostaining for PCNA and by autoradiography. Both fibrinoid necrosis of vessel walls and an accumulation of macrophages in the adventitia were occasionally demonstrated. This suggests that growth factors released by platelets and macrophages could be participating in the smooth muscle cell proliferation. Ang II–mediated hypertension has been previously reported to induce vascular smooth muscle cell proliferation in vivo.11,12 A component of the proliferative response to Ang II may occur independently of hypertension. Ang II induces vascular smooth muscle cells to express mRNA for a variety of protooncogenes and growth factors, including c-fos, c-myc, PDGF A-chain, and endothelin,14-32-33 and it may induce vascular smooth muscle cell proliferation in vitro under certain defined conditions.15 Several in vivo studies also suggest that Ang II mediates vascular cell proliferation independently of hypertension.13-34 The mechanism may involve the α-adrenergic system since the administration of prazosin, an α,-adrenergic inhibitor, to rats receiving Ang II inhibits aortic smooth muscle cell proliferation without decreasing blood pressure (E. VanKleef, unpublished results). Hypertension per se may also induce vascular smooth muscle cell proliferation, as evidenced by studies performed over 25 years ago in the deoxycorticosterone acetate (DOCA)–salt hypertensive rat.35

<table>
<thead>
<tr>
<th>TABLE 3. Comparison of Angiotensin II–Infused Rats With Vehicle–Infused Controls for the Degree of Vascular Proliferation at 14 Days in Small and Large Blood Vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Small blood vessels (&lt;54 μm i.d.)</td>
</tr>
<tr>
<td>Endothelium</td>
</tr>
<tr>
<td>Smooth muscle cells</td>
</tr>
<tr>
<td>Adventitial cells</td>
</tr>
<tr>
<td>Large blood vessels (&gt;54 μm i.d.)</td>
</tr>
<tr>
<td>Endothelium</td>
</tr>
<tr>
<td>Smooth muscle cells</td>
</tr>
<tr>
<td>Adventitial cells</td>
</tr>
</tbody>
</table>

Values for labeled cells are mean ± SD. Degree of vascular proliferation was assessed by [3H] thymidine labeling indexes.

FIGURE 3. Photomicrographs show blood vessels from control rats demonstrating minimal proliferation of the smooth muscle cells when immunostained for proliferating cell nuclear antigen (PCNA) at day 7 (panel a). In contrast, the small arterial vessels from angiotensin II (Ang II)–infused rats had numerous PCNA positive cells (panel b). In addition, an infiltration of macrophages (ED-1 positive cells) was present in the adventitial regions of the involved blood vessels in Ang II–infused rats (panel c). Biopsies at day 7; magnification × 400.
Angiotensin II (Ang II)–infused rats developed focal tubulointerstitial injury that was evident at 14 days, as shown by the area demarcated by the arrows in the low power (×125) micrograph in panel a. A higher power (×400) micrograph demonstrates dilated tubules filled with cast material, an arteriole with endothelial cell injury (arrow), and a mononuclear cell infiltrate in the interstitium (panel b). Although vehicle-infused control rats had only an occasional proliferating (proliferating cell nuclear antigen [PCNA] plus) tubular cell (panel c), Ang II–infused rats had marked tubular and interstitial cell proliferation in the areas of injury (PCNA positive cells identified by the dark nuclear stain) (panel d). Some cells were undergoing mitosis (arrows); these cells are only faintly PCNA positive (panel d). When the tissue was immunostained for type IV collagen, an increased deposition of type IV collagen in the areas of interstitial injury was demonstrated (panel f, marked by the asterisk) compared with controls (panel e). Day 14; magnification ×400 panels b–f.

Glomerular Changes

Given the profound proliferative response of the vascular smooth muscle cell to Ang II infusion, we examined whether the smooth muscle cell–like glomerular mesangial cell would demonstrate a similar proliferative response. In culture, mesangial cells morphologically resemble smooth muscle cells, express smooth muscle cell proteins, and contract in response to a variety of agonists (see Reference 36 for review). Like smooth muscle cells, there are reports that Ang II stimulates mesangial cell proliferation in culture and reports that it has no effect.37–39 However, in contrast to the marked proliferation of vascular smooth muscle cells observed in our study, the infusion of Ang II resulted in only a minimal increase in glomerular cell proliferation. Potentially, the lack of glomerular cell proliferation could be due to differences in the degree to which Ang II increases the pressure and tension on the systemic arterial vessel walls as compared against within the glomeruli, differences in the mitogenic response of mesangial cells versus smooth muscle cells to Ang II, differences in quantity and type of Ang II receptors, or differences in availability of Ang II.

To investigate differences in availability of Ang II, we examined the glomerular localization of the Ang II–degrading enzyme, aminopeptidase A,39 in Ang II–
infused and control rats. Glomerular aminopeptidase A was qualitatively increased in Ang II-infused rats. A similar increase in glomerular aminopeptidase A has been reported in other Ang II-dependent conditions, such as after saline diuresis or in the remnant kidney model. It is therefore possible that the concentrations of Ang II within glomeruli were lower than in the preglobular vasculature due to increased degradation of Ang II by glomerular aminopeptidase.

In addition to effects on cell proliferation, studies in cell culture suggest that Ang II may control synthesis of proteins that make up the contractile mass of smooth muscle. Our data indicate that similar specific effects may occur in the glomerulus. The effect is most pronounced for the mesangial cell in which a dramatic upregulation of α-smooth muscle actin occurred in response to Ang II. Previously we had demonstrated that the de novo expression of α-smooth muscle actin
expression by mesangial cells occurs in experimental glomerulonephritis and coincides with the onset of mesangial cell proliferation.\(^\text{17}\) a-Smooth muscle actin expression also correlates with mesangial cell proliferation in a variety of glomerular diseases in humans.\(^\text{40}\) However, in the current study a-smooth muscle actin expression by mesangial cells was quite impressive despite minimal glomerular cell proliferation. This suggests that mesangial cells may express a-smooth muscle actin independently of proliferation, possibly as a consequence of the direct effects of Ang II, hypertension, or both. In this regard, we have recently shown that a-smooth muscle actin expression by mesangial cells precedes the onset of glomerular cell proliferation in rats after 5/6 nephrectomy, a model of Ang II-dependent glomerular hypertension.\(^\text{25}\)

The functional consequence of the mesangial a-smooth muscle actin expression remains unknown. One may speculate that the upregulation of smooth muscle-associated contractile proteins may be an adaptive response of these cells to counter the increased tension and distending forces that accompany intraglomerular hypertension.\(^\text{31}\) Alternatively, it may represent a response by these cells to pharmacological as opposed to physiological concentrations of Ang II.

The expression of the muscle-associated intermediate filament protein, desmin, was also studied. In vivo, desmin is normally expressed by mesangial cells with variable expression by visceral glomerular epithelial cells.\(^\text{17}\) However, desmin expression was markedly increased in the visceral glomerular epithelial cells of Ang II-infused rats. Although the role of desmin filaments in epithelial cell function is not known, one may speculate that the increased desmin expression by the glomerular epithelial cell may reflect an adaptive response to the stretch-mediated shape change that occurs with glomerular hypertension and hypertrophy.\(^\text{42}\)

**Tubular Cell Changes**

Tubular cell injury and proliferation was focal and often limited to areas surrounding injured arterial vessels. It is not known at this time whether the tubular injury is the result of Ang II-mediated vasoconstriction and ischemia or whether the injury is a consequence of direct effects of Ang II, hypertension, or both. It is possible that Ang II may function as a growth factor. Renal tubular cells\(^\text{41,44}\) and renal interstitial cells\(^\text{45}\) have Ang II receptors. Although Ang II does not appear to be mitogenic for cultured rabbit or murine proximal tubule cells.\(^\text{41,46}\) Ang II does potentiate the proliferative response of these cells to epidermal growth factor.\(^\text{44b}\) Ang II may also induce hypertrophy of murine\(^\text{43}\) but not rabbit\(^\text{46}\) proximal tubular cells in vitro. The effect of Ang
II on distal tubule and collecting duct cells is less well known, although these cells also have Ang II receptors. It is interesting that the tubular cell proliferation primarily involved these latter cell types since, unlike the proximal tubule cells, these cells are negative for the Ang II-degrading enzyme aminopeptidase A.

It is also possible that the tubular cell proliferation is mediated by growth factors released by cells within the kidney. The observation in this study as well as in DOCA-salt hypertension that there frequently, though not invariably, was a gradient of proliferation spreading out from the blood vessels could be interpreted as evidence for the release of growth factors released by injured endothelial cells, smooth muscle cells, macrophages, or degranulating platelets at the site of vascular injury. A role for interstitial cells or tubular cells should also be considered.

Although numerous growth factors may be involved, we initially examined the effect of Ang II-mediated hypertension on PDGF B-chain mRNA expression. By in situ hybridization, an increase in PDGF B-chain mRNA was apparent in the areas of vascular and tubular injury, with expression by distal tubules and collecting ducts and interstitial cells, as well as smooth muscle cells within the media of the small arteries and arterioles. The observation that the tubule cells were producing PDGF B-chain mRNA suggests that PDGF may be functioning as an autocrine growth factor. However, whereas murine proximal tubule cell line (MCT cells) proliferate in response to PDGF in vitro, primary cultures of rabbit proximal tubule epithelial cells do not. Kartha et al. also have reported that BSC-1 cells, a monkey renal tubular cell line, produce PDGF B-chain mRNA and protein in culture but do not proliferate in response to PDGF, suggesting that any PDGF released by tubular cells may act solely as a paracrine growth factor. The observation that cultured renal interstitial cells proliferate in response to PDGF suggests that the PDGF released in the areas of interstitial injury in our model may have acted on this cell type.

**Interstitial Changes**

The infusion of Ang II was associated with interstitial cell proliferation, phenotype changes in which numerous interstitial cells expressed α-smooth muscle actin, and by the deposition of type IV collagen with fibrosis formation. In normal kidneys, the majority of interstitial cells lack markers characteristic of specific cell types and are usually labeled "fibroblasts." In Ang II-infused kidneys, these cells underwent phenotypic modulation with the induced expression of α-smooth muscle actin, a protein usually associated with smooth muscle cells. The observation that renal interstitial cells may express smooth muscle cell--like antigens has also been reported in the rabbit model of ureteral obstruction in which interstitial cells express numerous actinlike myofilaments and cross-react with anti-smooth muscle antibodies. The finding that renal interstitial cells acquire "smooth muscle--specific" proteins is reminiscent of the transient expression of α-smooth muscle actin by fibroblasts during wound repair, by parenchymal cells in experimental interstitial lung disease, and by mesangial cells in mesangial proliferative nephritis. Thus, it appears likely that the renal interstitial "fibroblast" becomes activated in Ang II--mediated injury, assuming characteristics of "myofibroblasts" in that these cells transiently proliferate, express α-smooth muscle actin, and secrete type IV collagen.

The last finding of interest was that Ang II--mediated hypertension induced significant but focal tubulointerstitial disease. Previous studies have emphasized the importance of tubulointerstitial disease as a risk factor for the progression to end-stage renal failure (see Reference 54 for review). Despite its potential importance, the pathogenic mechanisms responsible for the development of interstitial fibrosis remain unclear (see Reference 55 for review). The present study emphasizes that Ang II--mediated hypertension may be an important contributory factor and is consistent with previous studies demonstrating tubulointerstitial disease in rats with both Ang II--dependent and Ang II--independent hypertension, and in patients with primary hypertension--associated renal disease.

In conclusion, numerous and distinct effects of Ang II on the various renal cell populations were observed within 2 weeks in the rat, including a proliferative response of vascular, glomerular, tubular, and interstitial cells, as well as phenotypic modulation of glomerular mesangial and interstitial cells to express α-smooth muscle actin, consistent with their acquisition of smooth muscle cell--like features. The proliferative response was associated with increased expression of PDGF B-chain mRNA in the areas of tubulointerstitial injury.

Two important questions are generated by these studies. First, it is not known how much of the structural and phenotypic changes can be attributed to the consequences of hypertension as opposed to a direct effect of Ang II separate from hypertension. Second, the observation that Ang II is capable of inducing sustained hypertension, as opposed to many other hypertensive agents, raises the interesting possibility that the tubulointerstitial injury induced in the kidney by Ang II may account for this effect. Further studies are in progress to address these issues.

**Acknowledgments**

We thank Dr. William Couser and Dr. Robert Davidson for their valuable advice and suggestions; Zoe Jonak, Kelly Huddins, Michael Garvin, and Katherine Gordon for their excellent technical assistance; and Dr. Richard Zager for performing the blood urea nitrogen assays. We also thank Dr. G. Gabbiani, Dr. J. Lessard, Dr. F. Sendo, and Dr. M. Laprade for providing various monoclonal antibodies used in this study.

**References**


Renal injury from angiotensin II-mediated hypertension.
R J Johnson, C E Alpers, A Yoshimura, D Lombardi, P Pritzl, J Floege and S M Schwartz

doi: 10.1161/01.HYP.19.5.464

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/19/5/464

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Hypertension* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Hypertension* is online at:
http://hyper.ahajournals.org//subscriptions/