Effects of angiotensin II on regional afferent and efferent arteriole dimensions and the glomerular pole

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Received 26 July 1999; accepted in final form 10 March 2000

Denton, Kate M., Warwick P. Anderson, and Raja Sinniah. Effects of angiotensin II on regional afferent and efferent arteriole dimensions and the glomerular pole. Am J Physiol Regulatory Integrative Comp Physiol 279: R629–R638, 2000.—The diversity of renal arteriole diameters in different cortical regions has important consequences for control of glomerular capillary pressure. We examined whether intrarenal angiotensin II (ANG II; 0.1, 1, or 5 ng · kg⁻¹ · min⁻¹) in anesthetized rabbits acts preferentially on pre- or postglomerular vessels using vascular casting. ANG II produced dose-related reductions in afferent and efferent diameters in the outer, mid, and inner cortex, without effecting arterial pressure. Afferent diameter decreased more than efferent in the outer and mid cortex (P < 0.05) but by a similar extent in juxtamedullary nephrons (P = 0.58). Calculated efferent resistance increased more than afferent, especially in the outer cortex (127 vs. 24 units; 5 ng · kg⁻¹ · min⁻¹ ANG II). ANG II produced significant dose-related increases in the distance between the arterioles at the entrance to the glomerular pole in all regions. Thus afferent diameter decreased more in response to ANG II, but efferent resistance rose more due to smaller resting luminal dimensions.

The results also indicate that glomerular pole dimensions change in response to ANG II.

juxtamedullary, kidney, mesangium; renal vascular resistance; vascular casting

ANGIOTENSIN II (ANG II) has powerful effects on the renal microcirculation, causing potent constriction of the afferent and efferent arterioles and reduction of the glomerular filtration coefficient (4, 29). However, controversy still exists over several aspects of the roles of ANG II on the renal vasculature. The relative potency of ANG II on the two arterioles, particularly in the different regions of the cortex, still has not been definitively addressed. This is an important issue because of the potential for different effects on renal function, including glomerular filtration pressure, glomerular filtration rate (GFR), and renal blood flow. For example, the outer cortical glomeruli have a smaller surface area available for filtration than juxtamedullary glomeruli, and therefore differential vasoconstriction of the different arterioles in this region may reduce GFR less than vasoconstriction of the inner cortex. Similarly, glomerular capillary pressure depends on the relative effects of diameter changes on afferent and efferent arteriole vascular resistances. Furthermore, inner medullary flow is derived almost entirely from the efferent arteriolar drainage of the juxtamedullary glomeruli, and thus preferential constriction of the vessels of this region will have important consequences for the renal papilla, its concentrating ability, and its putative endocrine function (6).

It seems likely that some of the controversy surrounding the effects of ANG II on afferent and efferent arterioles can be explained on the basis of differences in experimental circumstances (4, 13, 14, 21, 29, 31) and on the functional difference between changes in diameter vs. changes in vascular resistance as we have previously argued (14). In our previous study (14), we measured vessel luminal diameters from renal vascular casts and showed a preferential decrease in outer cortical afferent arteriole diameter. When vascular resistance changes were calculated from these diameters using Poiseuille’s relationship, however, greater increases in efferent resistance were calculated compared with afferent resistance. We explained this apparent dichotomy between diameter and resistance change on the basis of differing sizes of the arterioles, because efferent arterioles have smaller diameters than afferent arterioles in the outer cortex (24) and resistance is inversely proportional to the fourth power of the radius (Poiseuille’s relationship). That is, the smaller resting diameter of the efferent arteriole amplified the hemodynamic effects of this vessel in response to ANG II (and possibly to other vasoconstrictors), helping to explain how the efferent vessels can exert substantial hemodynamic effects (14) despite their poor supply of contractile elements.

In our previous study, however, ANG II was administered intravenously, and consequent changes in arterial pressure may have confounded the results because of autoregulatory responses in the afferent arterioles. These responses may have led to an underestimation of the direct effects of ANG II on the afferent arteriole. Our findings also have been contested by others, arguing against our conclusions of a preferential increase in postglomerular resistance (17).

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Therefore, we have infused graded doses of ANG II directly into the renal artery without systemic effects, and with the technique of vascular casting, we measured the renal afferent and efferent arteriole dimensions in the outer, mid-, and juxtamedullary cortical regions and calculated the total contribution to renal resistance made by each vessel. In theory, the diversity of the diameters of the afferent and efferent arterioles in the different cortical regions has important consequences for the control of glomerular capillary pressure. Therefore, we tested the hypotheses that ANG II preferentially increases resistance in the smaller efferent arterioles and that regionally ANG II preferentially increases vascular resistance in the outer compared with the inner cortex.

In further analysis, we tested whether ANG II affects the dimensions of the glomerular vascular pole. Although there are many reports of ANG II-mediated mesangial contraction in vitro, the consequences for glomerular vascular structure and function in vivo remain unclear. Elger and colleagues (16) have recently reported the provocative idea that the vascular pole of the glomerulus, including the extraglomerular mesangium, may have several roles in the regulation of glomerular hemodynamics. They suggest that these roles may include structural support of the glomerulus, a direct functional influence of the afferent on the efferent arteriole, the presence of a specific shear stress receptor located in the intraglomerular portion of the efferent arteriole, and a role in interstitial fluid leakage. Therefore, the current study also has examined whether there are changes in the architecture of the glomerular vascular pole in response to ANG II. In particular, we have studied the possibility not previously considered that contraction of the extraglomerular mesangium may result in changes in the arterioles as they leave the glomerulus; for example, contraction could elongate the extraglomerular mesangial base or broaden it (changing the angles at which the vessels exit the glomerulus) with consequent hydraulic effects. Accordingly, we have measured the “interarteriole distance,” the distance between the afferent and efferent arteriole following graded doses of ANG II.

METHODS

Animals and Preparation

Four groups of six rabbits were studied with one of the following four infusions directly into the renal artery: 1) vehicle infusion (0.34 ml/min saline), 2) 0.1 ng·kg\(^{-1}\)·min\(^{-1}\) ANG II (ANG II human peptide; Sigma Chemical, St. Louis, MO), 3) 1 ng·kg\(^{-1}\)·min\(^{-1}\) ANG II, or 4) 5 ng·kg\(^{-1}\)·min\(^{-1}\) ANG II.

Male rabbits from a colony crossbred from local and New Zealand White strains were used in this study (mean body wt 2.24 ± 0.03 kg, range 2.0–2.5 kg). Catheters (polyvinyl chloride (PVC); 0.58 mm ID, 0.96 mm OD) were placed in the central ear artery and vein using local anesthetic (0.5% lignocaine; Astra Pharmaceutical, Singapore). Mean arterial pressure was measured continuously throughout the experiment.

The rabbits were anesthetized with pentobarbital sodium (60 mg/kg iv bolus plus 0.2 mg·kg\(^{-1}\)·min\(^{-1}\) iv; Nembutal, CEVA, Paris, France) and ventilated. Saline (0.15 ml·kg\(^{-1}\)·min\(^{-1}\)) was infused intravenously throughout the experiment. A left flank incision was made, and the skin and muscle were retracted to expose the left kidney. A catheter was passed into the left renal vein (PVC; 0.5 mm ID, 1.0 mm OD) via a side branch. Infusions into the left renal artery were made via a catheter (PVC; 0.2 mm ID, 0.5 mm OD) placed in a side branch that led directly into the renal artery (saline, 0.34 ml/min was infused into this catheter). The left kidney was denervated (10). A catheter was inserted into the left ureter (PVC; 0.5 mm ID, 1.0 mm OD). In preparation for perfusion fixation at the end of the experiment, a large bore catheter was inserted into the lower aorta (PVC; 2.0 mm ID, 3.00 mm OD) and the abdominal aorta above the renal arteries was cleared to facilitate later clamping. On completion of the surgery, 0.33 μCi/ml [\(^{3}H\)julin (Amersham International) was added to the intravenous infusion and 1 h was allowed for equilibrium to be reached before measurements commenced.

Experimental Protocol

Measurements of renal hemodynamics and function were made over a period of 1 h and comprised three clearance periods. Each period consisted of a 10-min urine collection, with arterial and renal venous blood samples and hematocrit taken at the midpoint. During the first period baseline measurements were made, the second period was taken 20 min after converting-enzyme inhibition (2 mg/kg iv bolus enalaprilat; Merck, Sharpe and Dohme, Rahway, NJ), and the third period 10 min after one of the four previously mentioned infusions was started. GFR was calculated as the clearance of [\(^{3}H\)julin, filtration fraction as the arteriovenous concentration difference of [\(^{3}H\)julin, and renal blood flow as GFR divided by filtration fraction, corrected for hematocrit. Renal vascular resistance was calculated as the mean arterial pressure divided by renal blood flow.

Vascular Casts

Once all the measurements were completed the kidneys were immediately perfusion fixed, at which time ANG II had been infused for 20 min. Perfusion fixation was performed using a method previously described (1, 14). Briefly, 1 liter of 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3–7.4) at room temperature was perfused retrogradely through the distal aorta. The pressure head in the perfusion apparatus was 150 mmHg, and pressure as the catheter entered the aorta was measured to be ~100 mmHg. The upper aorta was clamped above the kidneys, and the vena cava was vented as soon as perfusion of the fixative commenced. Simultaneously, an overdose of anesthetic was injected via the central ear vein. After completion of the perfusion fixation, the casting resin (Batsons 17; Polysciences, Warrington, PA) prepared in the ratio 4:1:0.1 monomer, catalyst, and promoter, was injected through the aortic catheter at the same pressure as the fixative was perfused. The renal vessels were then clamped, and the resin was allowed to harden in situ for 1 h. The kidneys were then removed and placed in 2.5% glutaraldehyde overnight to allow polymerization of the resin to complete. The kidneys were then sliced into five pieces, and a portion (~5 × 3 × 3 mm) of each was taken to be prepared for scanning electron microscopy. The portions of the kidney were placed in 20% KOH for 72 h to dissolve the tissue from around the vascular cast. The casts were then rinsed in distilled water and placed in 5% sodium hypochlorite for 1 h.
The clean vascular casts were then dried, mounted, and gold coated before examination in a Phillip 615 scanning electron microscope at 20 kV.

**Vessel Diameters and Lengths**

Diameters of interlobular, afferent, and efferent vessels from outer, midcortical, and juxtamedullary glomeruli were measured from scanning electron micrographs, final magnification ×500 (Fig. 1). The following criteria were used to classify the three classes of glomeruli: 1) The afferent arterioles of outer cortical glomeruli originated from a terminal branch of an interlobular artery, and the efferent arteriole ascended to the cortical surface. 2) Afferent arterioles from midcortical glomeruli branched from interlobular arteries that proceeded on further through the cortex and the efferent arterioles supplied neither the outer cortical region nor the medulla. 3) Juxtamedullary glomeruli had efferent arterioles that descended into the renal medulla. In each group, six rabbits were studied; only vascular casts of glomeruli with all vessels visible and paired measurements possible were included. Approximately 140 outer cortical glomeruli, 180 midcortical, and 60 juxtamedullary glomeruli were included in the analysis. Micrographs were coded and randomized before measurements were made using a digitizing tablet (Summagraphics; resolution 100 lines/mm, accuracy ± 0.25 mm, GTCO Calcorp) and the MEASURE program (Capricorn Scientific Software, Victoria, Australia). Interlobular artery luminal diameters were measured between the midpoints of the afferent and efferent arterioles in the region of the glomerulus (Fig. 1), and a mean diameter was calculated. The sites of entry and exit were pinpointed as previously described (14). The length of the afferent arteriole from the interlobular artery to the glomerulus was measured. Efferent arteriole length was measured from the glomerulus to the first branching point. However, the terminal portion of the efferent arteriole was sometimes obscured by the surrounding peritubular capillary network or in some cases broken during processing, and, therefore, reported mean efferent length is necessarily an underestimation. Nephrons in which efferent arterioles could not be measured to the first branching point were only included if at least a 100-μm length was visible.

**Glomerular Dimensions**

The mean diameter of each glomerulus was determined as the mean of the measured maximum and minimum diameters. Glomerular tuft volumes were calculated using the equation $V = (4/3) \pi r^3$.

The interarteriole distance, the linear distance between the afferent and efferent arterioles in the region of the glomerular pole, also was determined. This distance was measured between the midpoints of the afferent and efferent arteriole at the entrance to the glomerulus; this position was previously designated during the vessel diameter measurements as 0 μm (Fig. 1).

**Relative Resistance Calculations**

The relative resistance ($R$) of a vessel can be calculated according to Poiseuille's equation $R = 8 n l/r^4$, where $n$ is the viscosity of the fluid, $l$ the length of the vessel, and $r$ the radius of the vessel. Relative vessel resistance per unit length ($R_l = l/r^4$) as a function of vessel diameter was calculated for the interlobular artery. Resistance per total arteriole length ($R_T = l/r^4$) was calculated for the afferent and efferent arterioles using a mean radius for each vessel. Viscosity was assumed to be constant in all calculations. The efferent-to-total arteriole resistance ratio ($R_T/R_T$) was calculated as $R_T$ equals the sum of the afferent ($R_A$) and efferent ($R_E$) arteriole resistances in the different cortical regions (the resistances are per total vessel length).

**Statistics**

All data are reported as means ± SE. A mean value for each variable measured was calculated for each rabbit; all statistical comparisons were performed on six animals in each of the four groups. The physiological data were analyzed using a one-way ANOVA followed by appropriate partitioning of the data to test for dose-related changes to the ANG II infusion. The morphometric data were analyzed using a two-way ANOVA. For the vessel profile data (see Fig. 3) the main effects were vessel (vessel segment, i.e., 0, 25, 50, 75, or 100 μm) and dose (vehicle, 1 or 5 ng·kg$^{-1}$·min$^{-1}$ ANG II) with interaction vessel segment × dose. For the mean afferent and efferent diameters and resistance data (see Fig. 4 and Table 1) the main effects were region (outer, midcortical, or juxtamedullary) and dose (vehicle, 1 or 5 ng·kg$^{-1}$·min$^{-1}$ ANG II) with interaction region × dose. $P < 0.05$ was considered to be statistically significant.

**RESULTS**

**Physiological Data**

Baseline mean arterial pressure [69 ± 3, 65 ± 5, 67 ± 3, 67 ± 4 mmHg; not significant (NS)], renal vascular resistance (2.5 ± 0.3, 2.8 ± 0.9, 1.9 ± 0.3, 1.8 ± 0.2 mmHg·ml$^{-1}$·min$^{-1}$; NS), renal blood flow...
Table 1. Outcome of two-way analysis of variance for data depicted in Fig. 4

<table>
<thead>
<tr>
<th>Arteriole</th>
<th>$P_{\text{Region}}$</th>
<th>$P_{\text{Dose}}$</th>
<th>$P_{\text{Region} \times \text{Dose}}$</th>
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<tr>
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<td>$&lt;0.01$</td>
<td>$&lt;0.05$</td>
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<tr>
<td>Resistance</td>
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<td>$&lt;0.01$</td>
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<tr>
<td>Cortex</td>
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<tr>
<td>Diameter</td>
<td>$=0.64$</td>
<td>$&lt;0.01$</td>
<td>$=0.59$</td>
</tr>
<tr>
<td>Resistance</td>
<td>$&lt;0.01$</td>
<td>$&lt;0.01$</td>
<td>$=0.62$</td>
</tr>
</tbody>
</table>

$P$ values for differences between cortical regions ($P_{\text{Region}}$) and for the effects of increasing dose of ANG II ($P_{\text{Dose}}$) were derived from $F(45,2)$ and the interaction between cortical region and increasing dose of ANG II ($P_{\text{Region} \times \text{Dose}}$) was derived from $F(30,1)$. $P$ values for differences between arteriole ($P_{\text{Arteriole}}$) and for the effects of increasing dose of ANG II ($P_{\text{Arteriole} \times \text{Dose}}$) and the interaction between arteriole and increasing dose of ANG II ($P_{\text{Arteriole} \times \text{Dose}}$) was derived from $F(30,2)$.

(30 ± 4, 31 ± 5, 39 ± 6, 39 ± 4 ml/min; NS), GFR (3.1 ± 0.3, 3.3 ± 0.6, 3.7 ± 0.5, 3.5 ± 0.3 ml/min; NS), and filtration fraction (0.18 ± 0.01, 0.18 ± 0.20, 0.16 ± 0.02, 0.16 ± 0.02%; NS) were similar in all groups before the intrarenal infusions (vehicle, 0.1, 1, and 5 ng kg$^{-1}$ min$^{-1}$ ANG II infusion, respectively).

There were no significant differences in arterial pressure in any of the four groups (vehicle, 0.1, 1, or 5 ng kg$^{-1}$ min$^{-1}$ ANG II into the renal artery) at the time of fixation of the kidney (Fig. 2). Renal blood flow decreased by ∼17, 35, and 65% compared with vehicle during infusion of ANG II at 0.1, 1, and 5 ng kg$^{-1}$ min$^{-1}$, respectively ($P < 0.005$, Fig. 2) with significant increases in renal vascular resistance ($P < 0.001$, Fig. 2). Filtration fraction was 0.17 ± 0.01% in the vehicle group, rising to 0.20 ± 0.01, 0.27 ± 0.02, and 0.37 ± 0.03 in the 0.1, 1, and 5 ng kg$^{-1}$ min$^{-1}$ ANG II groups, respectively ($P < 0.001$, Fig. 2). In contrast, there was no significant dose-related change in GFR (Fig. 2). GFR values were directly compared between the vehicle and 5 ng kg$^{-1}$ min$^{-1}$ ANG II groups (ANOVA, followed by 100–1 partition), no significant difference was found ($P = 0.78$).

Vessel Luminal Diameters and Calculated Relative Resistances

No significant effects were seen in any variable measured in the 0.1 ng kg$^{-1}$ min$^{-1}$ ANG II group, and for clarity the results from this group are not presented further.

Regional differences in afferent and efferent luminal diameters. Afferent and efferent arteriole luminal diameters at 0, 25, 50, 75, and 100 μm from the glomerulus for outer, midcortical, and juxtamedullary glomeruli are depicted in Fig. 3 (see also Fig. 1). Afferent arterioles were seen to taper toward the glomerulus and opened out as they branched to form the glomerular capillaries. The afferent arteriole was narrowest at 25 μm from the glomerulus, irrespective of treatment group. The efferent arterioles tapered as they moved away from the glomerulus.

Mean afferent and efferent arteriole luminal diameters for outer, midcortical, and juxtamedullary glomeruli are depicted in Fig. 4. In the vehicle group, there was a progressive increase in both afferent and efferent luminal diameters from outer to inner cortex, afferent diameters being 15.0 ± 0.8, 16.1 ± 1.1, and 20.0 ± 2.8 μm and efferent diameters being 9.5 ± 0.2, 10.8 ± 0.3, and 18.8 ± 2.6 μm for the outer cortical, midcortical, and juxtamedullary glomeruli, respectively. In the outer and midcortical glomeruli, the diameters of afferent arteriole were significantly greater
than their efferent counterparts \((P < 0.001)\), but for juxtamedullary glomeruli, efferent and afferent arterioles were similar in size \((P = 0.62)\).

The length of the afferent arteriole did not differ significantly in the outer cortical, midcortical, and juxtamedullary regions, averaging \(113.0 \pm 10.1\), \(100.1 \pm 6.9\), and \(111.5 \pm 19.0\) \(\mu\)m, respectively. Similarly, efferent arteriolar lengths also were not significantly different in the three cortical regions \((125.6 \pm 13.1\), \(150.3 \pm 11.5\), and \(159.5 \pm 38.5\) \(\mu\)m, respectively).

**Effects of increasing dose of ANG II on afferent and efferent arteriole dimensions.** Afferent arteriole luminal diameter decreased in response to the 1 and 5 ng \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) ANG II infusions by \(-1.9\) and \(-3.3\) \(\mu\)m in the outer cortical region, \(-0.8\) and \(-3.5\) \(\mu\)m in the midcortical region, and \(-4.9\) and \(-5.4\) \(\mu\)m in the juxtamedullary glomeruli, respectively (a significant dose-related decrease, \(P < 0.005\), Fig. 4 and Table 1). The responses to ANG II were similar in the three cortical regions (Fig. 4 and Table 1), and there was no evidence of focal constriction (i.e., no statistically significant interaction between vessel segment and dose; Fig. 3).

There was also a significant dose-related decrease in efferent arteriole diameter in response to ANG II \((P < 0.01\), Fig. 4 and Table 1). The response was significantly greater in the juxtamedullary efferent arterioles than the outer or midcortical glomeruli \((P < 0.05\) for the interaction between the effects of dose and region, Fig. 4 and Table 1). Comparing regional responses there were reductions in efferent arteriole luminal diameter of \(-0.3\) and \(-0.7\) \(\mu\)m in the outer cortical region, \(-0.5\) and \(-0.7\) \(\mu\)m in the midcortical, and \(-3.5\) and \(-6.7\) \(\mu\)m in the juxtamedullary glomeruli, in response to 1 and 5 ng \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) ANG II infusions, respectively.

There were no significant changes in the lengths of either the afferent or efferent arteriole in response to ANG II (data not shown).
Effects of increasing dose of ANG II on calculated relative arteriole resistances. The relative resistances given in this section were calculated per total measured length of each vessel, i.e., $R_i = l/l' R^4$. Relative afferent arteriole resistance rose on average by $11.8 \times 10^{-3}$ and $24.3 \times 10^{-3}$ units in the outer cortical region, $3.8 \times 10^{-3}$ and $25.2 \times 10^{-3}$ units in the midcortical, and $14.4 \times 10^{-3}$ and $10.1 \times 10^{-3}$ units in the juxtamedullary glomeruli, in response to 1 and 5 ng $\cdot$ kg$^{-1} \cdot$ min$^{-1}$ ANG II infusions, respectively ($P < 0.005$, Fig. 4). Similar changes occurred in calculated relative efferent arteriole resistance ($P < 0.01$, Fig. 4). The increase in calculated efferent arteriole relative resistance in the outer cortical regions was greater than in the other regions ($P < 0.05$, for the dose-region interaction, Fig. 4 and Table 1), efferent resistance increasing by $51.1 \times 10^{-3}$ and $127.5 \times 10^{-3}$ units in the outer cortical region, $19.0 \times 10^{-3}$ and $22.6 \times 10^{-3}$ units in the midcortical, and $13.1 \times 10^{-3}$ and $25.2 \times 10^{-3}$ units in the juxtamedullary glomeruli, in response to 1 and 5 ng $\cdot$ kg$^{-1} \cdot$ min$^{-1}$ ANG II infusions, respectively.

The $R_e/R_T$ for the three cortical regions in response to ANG II are given in Fig. 5. This ratio was significantly less in the juxtamedullary region ($P < 0.001$), but no significant effect of ANG II was observed in any region. This lack of change in $R_e/R_T$ in response to ANG II is addressed in the DISCUSSION.

**Interlobular artery.** In the vehicle group there were significant regional differences among the interlobular artery luminal diameters ($25.6 \pm 1.7$, $44.5 \pm 4.0$, and $67.1 \pm 10.1 \mu m$ in the outer, midcortical, and juxtamedullary regions, respectively, $P < 0.001$, Fig. 6), indicating that the interlobular arteries tapered as they moved from the inner to the outer cortical regions. A significant dose-related decrease in interlobular artery luminal diameter in response to ANG II was only associated with the portion of the interlobular artery supplying outer cortical glomeruli (Fig. 6; $P < 0.005$). The length of the interlobular artery could not be measured, but calculation of relative resistance per unit length showed a significant increase in resistance in the outermost branches of the interlobular arteries in response to ANG II (Fig. 6; $P < 0.001$). These relative resistance values per micrometer for the interlobular were compared with equivalent per micrometer resistance values for the afferent arteriole. In the outer cortical region, the interlobular artery was found to exert a resistance equivalent to about 15% of that exerted by afferent arteriole. Calculated relative resistance per micrometer in the outermost branches of the interlobular arteries were $2.0 \pm 0.3 \times 10^{-5}$, $3.8 \pm 0.8 \times 10^{-5}$, and $9.8 \pm 3.1 \times 10^{-5}$ units, compared with afferent arterioles from outer cortical glomeruli, which were $13.8 \pm 2.8 \times 10^{-5}$, $27.8 \pm 8.0 \times 10^{-5}$, and $42.0 \pm 9.9 \times 10^{-5}$ units in the vehicle, 1 and 5 ng $\cdot$ kg$^{-1} \cdot$ min$^{-1}$ ANG II infusion groups, respectively. The larger diameter of the interlobular artery in the mid- and inner cortical regions meant that in these regions the interlobular arteries were contributing a minor portion to preglomerular resistance compared with the afferent arterioles (1–5%).

**Effects of Increasing ANG II Dose on Glomerular Dimensions**

**Glomerular tuft volumes.** Glomerular tuft volumes were progressively larger from the outer to inner cortex, being $775 \pm 82 \times 10^{3}$, 1,028 $\pm 106 \times 10^{3}$, and 1,584 $\pm 102 \times 10^{3} \mu m^3$ in the outer, midcortical, and juxtamedullary glomeruli, respectively ($P < 0.001$). The glomerular volumes were $737 \pm 48 \times 10^{3}$, 1,069 $\pm 69 \times 10^{3}$, and 1,551 $\pm 138 \times 10^{3} \mu m^3$ in the group that received 1 ng $\cdot$ kg$^{-1} \cdot$ min$^{-1}$ ANG II and 839 $\pm 49 \times 10^{3}$, 1,068 $\pm 94 \times 10^{3}$, and 1,418 $\pm 109 \times 10^{3} \mu m^3$ in the group that received 5 ng $\cdot$ kg$^{-1} \cdot$ min$^{-1}$ ANG II in the outer, midcortical, and juxtamedullary glomeruli, respectively. Statistical analysis indicated no dose-re-

![Graph](image)
The effects of ANG II were not uniform throughout the cortex. There were greater reductions in afferent than efferent arteriole luminal diameters in outer and midcortical glomeruli, but similar decreases in afferent and efferent arteriole luminal diameters were observed in juxtamedullary glomeruli. Yet, despite this apparent preferential afferent effect of ANG II on diameter in the majority of glomeruli, filtration fraction rose and GFR was unchanged during the infusions of ANG II. These hemodynamic measurements are in accord with many previous studies. We hypothesize that this apparent dichotomy can be explained on the basis of differences in the diameters of the afferent and efferent arterioles and hemodynamic events consequent on this. As argued previously (14), the smaller efferent arteriole exerts a proportionally greater resistance in response to any given change in radius compared with the larger afferent arteriole. A diagrammatic representation of this can be found in the study by Denton et al. (14).

Anatomically, afferent and efferent arterioles are of progressively larger diameters from the outer to the inner cortex as shown in this study and a previous study (24). The efferent vessels are smaller than the afferent arterioles in the outer and midcortex but are of similar diameters in the juxtamedullary glomeruli. Changes in diameter may be translated into changes in resistance to flow using Poiseuille’s relationship in which resistance is inversely proportional to the fourth power of the radius. Thus for the outer cortical efferent arterioles, which were the smallest arteriole studied, ANG II caused only a small decrease in its diameter (≈1 μm), but this resulted in the greatest increase in resistance to flow regionally (Figs. 3 and 4). This may be contrasted with the larger juxtamedullary efferent arteriole. The diameter of this arteriole decreased markedly in response to ANG II (≈6 μm), but calculated vascular resistance rose by only a small amount (Figs. 3 and 4). Outer and midcortical glomeruli account for ~90% of all glomeruli, and thus overall there was a preferential increase in renal efferent resistance. As indicated above, these calculated resistance changes reflect the observed physiological effects of decreased renal blood flow, increased filtration fraction, and maintenance of GFR in response to these doses of ANG II.

Our previous report (14) that ANG II caused a preferential increase in efferent resistance in outer cortical glomeruli (now also shown to be the case for the midcortical arterioles) had been disputed. Endlich and Steinhausen (17) contended that the greater decrease in afferent diameter resulted in a greater percentage increase in afferent resistance, as indeed it did, and that this represented a preferential increase in preglomerular resistance. They went on to say that our morphometric analysis was in conflict with micropuncture studies (17). We disagreed, and in the current study we have designed the experiment to avoid some methodological problems of the previous study. Thus we avoided confounding autoregulatory responses by using intrarenal ANG II infusions, we examined the effects of ANG II over a range of doses, and we documented the actual length of each vessel. We also have studied the responses of the interlobular arteries. The present results fit with the results from micropuncture studies in superficial glomeruli, which have shown that ANG II caused a greater increase in efferent resistance, and this resulted in an increased single nephron filtration fraction and glomerular capillary pressure and maintenance of GFR (7, 8, 27). Another reason for the difference in opinion between ourselves and Endlich and Steinhausen (17) may have resulted from their interpretation of afferent resistance in our study being the same as preglomerular resistance. In micropuncture studies, preglomerular resistance and afferent resistance are often used synonymously. How-
However, in our morphometric study, afferent resistance equates to the resistance of the afferent arteriole alone, as calculated using Poiseuille’s relationship. In terms of the ratio of afferent to efferent resistance therefore our results and those of micropuncture experiments are not directly comparable, because in our study only a proportion of pre- and postglomerular resistance was measured. In micropuncture studies, renal vascular resistance is distributed 60:40 in the pre- and postglomerular vasculature; in our current study, resistance was distributed 20:80 in the afferent and efferent arterioles; clearly preglomerular resistance and afferent resistance are not equivalent. A large proportion of preglomerular resistance resides in the larger renal vessels (e.g., interlobular arteries) (5, 20). Whereas these larger vessels do not contribute substantially to changes in resistance, as shown in this study, compared with the afferent arteriole, they do contribute a large proportion of the resistance at rest, with alterations in afferent resistance being responsible primarily for physiological changes in preglomerular resistance (28). Measured changes in interlobular luminal diameters in response to ANG II in this study support this conclusion. Therefore, in terms of the changes in glomerular capillary pressure, it is the absolute increase that is important rather than the percent change in afferent and efferent resistances. In this morphological study of the arterioles only, the \( R_{g}R_{T} \) does not reflect the consequences for glomerular capillary pressure for the reasons given above.

The arterioles of the juxtamedullary glomeruli, about 10% of the total glomeruli number (24), show distinct differences to the arterioles elsewhere in the cortex as shown here and elsewhere (9, 23, 31). Both afferent and efferent arterioles are larger than elsewhere in the kidney and exert lower vascular resistance at rest. As well, the efferent arteriole is little different in diameter to the afferent. These juxtamedullary glomeruli are important because they supply blood to the renal medulla and hence the greater afferent and efferent arteriole diameters of these glomeruli will have physiological significance, possibly tending to result in relative protection of medullary vs. cortical flow. That is, the smaller diameters of the resistance vessels of the outer cortex may result in a greater increase in resistance in response to increasing ANG II levels than for the juxtamedullary glomeruli, thus conserving medullary flow relative to cortical flow (18).

ANG II decreases the glomerular filtration coefficient (8, 30), and this is thought to be due to contraction of mesangial cells, resulting in a decrease in filtration surface area. However, although ANG II is known to contract mesangial cells in culture, most studies that have examined the effects in vivo have not measured a decrease in the capillary surface area (2, 14, 19, 21, 30). In this study in which the ANG II was infused directly into the renal artery without changes in arterial pressure, we found no significant difference in the glomerular tuft volumes during ANG II infusion, providing further evidence against ANG II-mediated changes in glomerular capillary surface area.

A novel finding of the present study is that ANG II changed the geometry of the glomerular pole. The mesangium extends beyond the glomerulus (16), but the role of this extraglomerular mesangium has been little studied. Recent studies describing the three-dimensional structure of the glomerular mesangium (22) and glomerular pole region (16) have suggested to us that the drawing down (contraction) of the mesangium into the extraglomerular region may have consequences for glomerular dynamics. We therefore examined the glomerular pole region in this study and noted that the size of the open space between the afferent and efferent vessel casts varied considerably. This space houses the extraglomerular mesangium before digestion of the tissue from the casts. We therefore measured (blinded) the interarteriole distance, the linear distance between the afferent and efferent arterioles at the entrance to the glomerulus, at previously determined points for all the glomeruli studied above. The results showed the origins of the afferent and efferent arterioles were further apart in the ANG II-treated groups and this was dose-related. Because the space between the arterioles in this region houses the extraglomerular mesangium (16), the increase in the interarteriole distance possibly indicates that the downward contraction of the glomerular mesangial tree has led to distension in this region. This might be associated with tugging on the recently identified mesangial loops (22), causing changes in flow distribution within the glomerular capillaries and a decrease in the effective filtration surface area. Elger et al. (16) reported that the confluence of tributaries forming the efferent arteriole occurs deep within the glomerulus, and we also observed the efferent arteriole to be always fully formed as it left the glomerular tuft in the present study. Therefore, it is also possible that broadening of the mesangium in this pole region may alter the dimensions of that portion of the intraglomerular efferent arteriole segment. However, at the point we measured the interarteriole distance, just as the vessels entered Bowman’s capsule, we saw no evidence of greater narrowing of the efferent arteriole. Another possibility is that the angles at which the vessels enter and exit the glomerulus have changed, which could lead to hydraulic consequences. The functional consequences of this increase in the interarteriole diameter remains to be determined.

Vascular casting is a technique that involves fixation of the tissue and then filling the vasculature with resin. Some artifact associated with these procedures is inevitable. The fixation and casting methods used in this study and the validation for their use have been discussed previously (14, 15, 25, 26). In the current study, the diameters of the renal arterioles measured from the vascular casts are comparable to those previously reported using several techniques, including vascular casting (9, 11, 14, 23, 33). The preparation described here, using in vivo whole kidney measurements in conjunction with vascular casting, allows the opportunity to correlate physiological and morphological effects of ANG II. Renal function in this anesthetized
preparation has been shown to be comparable to that reported for conscious rabbits (12), and in this study physiological values were within the normal range for conscious rabbits. The limitations of the technique of vascular casting was apparent in this study from the lowest dose of ANG II used (0.1 ng \cdot kg^{-1} \cdot min^{-1}). Although this dose produced a significant fall in renal blood flow of about 17%, the effects on the vasculature were too small to be detected.

Although the changes seen in this study are due to the intrarenal infusion of ANG II (without changes in arterial pressure) and experiments were performed under conditions of acute converting-enzyme inhibition and renal denervation, they are not necessarily due to a direct action of ANG II. The responses of the arterioles reported here are different from those seen in isolated arteriole preparations (23), in which efferent diameters are seen to decrease more than afferent arterioles. This suggests that in this in vivo preparation the response to ANG II may be dependent in part on interactions with other factors such as bradykinin, prostaglandin, nitric oxide, endothelin, or tubuloglomerular feedback (29). Renal denervation might have had different effects on resting afferent and efferent resistance; however, previous studies would suggest that this would not be the case (32).

In conclusion, increasing doses of intrarenal ANG II caused a progressive decrease in renal blood flow, with a concomitant increase in filtration fraction resulting in maintenance of GFR. Measurement of arteriole diameter changes indicated that these results were explained by there being a significantly greater increase in relative efferent arteriole resistance in the outer and midcortical arterioles compared with afferent arteriolar resistance, even though there were greater absolute reductions in afferent arteriole diameters. Thus whereas the two arterioles were of similar length in all regions of the kidney, the significantly smaller diameters of the efferent arterioles amplifies the effects of vascular resistance of ANG II-mediated diameter changes. The arterioles of the juxtamedullary glomeruli exhibited markedly different responses to the rest of the cortical vessels. This study also reports new data indicating that ANG II may have significant effects on dimensions of the glomerular vascular pole. We found an ANG II dose-related increase in the distance between the afferent and efferent arteriole at their junction with the pole of the glomerulus.

**Perspectives**

There are important hemodynamic consequences of the regional differences in diameters of the afferent and efferent arterioles. The smaller diameter of the efferent arteriole in the outer and midcortical glomeruli allows for fine control of glomerular capillary pressure and thus GFR. In the juxtamedullary glomeruli that supply flow to the medulla, ANG II has less effect on vascular resistance due to the larger size of the vessels. This may result in renal medullary flow being less affected by ANG II, and presumably other vasoconstrictor agents, providing a structural basis for the different regulation of medullary and cortical blood flow in some circumstances. Overall, the results emphasize the importance of structure in the functional responses of the renal vasculature to stimuli. Afferent and efferent arterioles are of differing resting diameters and lengths depending on their location in the cortex, and this has important consequences because resistance is inversely proportional to the fourth power of the radius. Thus many vasoconstrictor agents will tend to preserve GFR more than flow by this structurally determined greater effect on the efferent arteriole. The potential significance of the other main finding of this study, that ANG II increases the distance between the afferent and efferent arterioles at the points where they connect to the glomerulus, remains to be determined.

We acknowledge the technical assistance of Kee Wei Hua and Rebecca Gribben. Enalaprilat was a gift from Merck, Sharpe and Dohme (Asia), Singapore. This research project was supported by a National University of Singapore Grant RP910477 and the National Health and Medical Research Council of Australia Grant 970246.

**REFERENCES**


