Distinct Modulation of Superficial and Juxtamedullary Arterioles by Prostaglandin in Vivo

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Renal afferent (AFF) and efferent arteriolar (EFF) responsiveness to angiotensin II (ANG II) in superficial and juxtamedullary nephrons in vivo remains undetermined, nor has it been clarified what role intrarenal autocrines/paracrines play in modulating the renal microvascular response. The present study characterized the responsiveness to ANG II (1–30 ng/kg/min) of AFF and EFF of canine superficial and juxtamedullary nephrons under pentobarbital anesthesia, using intravital CCD-videomicroscopy that allowed direct in vivo visualization of the renal microcirculation. Furthermore, the effect of prostaglandins (PG) and nitric oxide (NO) on ANG II-induced tone was examined. In superficial nephrons, ANG II induced a similar dose-dependent constriction of both AFF (46 ± 5% constriction) and EFF (53 ± 3%). In juxtamedullary arterioles, ANG II induced a dose-dependent constriction of EFF, whereas AFF responses were diminished (17 ± 4% vs. 37 ± 4% at 10 ng/kg/min). The PG inhibition by indomethacin enhanced the ANG II-induced constriction of juxtamedullary AFF, whereas no augmentation was observed in other arterioles. In contrast, NO inhibition by nitro-arginine methyl ester (L-NAME) enhanced the ANG II-induced constriction, with greater augmentation in juxtamedullary AFF and EFF. Finally, renal interstitial PG and nitrite/nitrate contents were greater in the medulla than the superficial cortex under basal and ANG II-stimulated conditions. Taken together, the results of the intravital CCD-videomicroscopy reveal that the renal microvascular action of ANG II had both zonal (juxtamedullary vs. superficial nephrons) and segmental (AFF vs. EFF) heterogeneity under the present experimental conditions. This heterogeneity was associated with a difference in the intrarenal production of prostaglandin E2 (PGE2) and NO; PGE2 contributed to segmental and zonal differences whereas NO was responsible for the zonal heterogeneity in arteriolar responsiveness. (Hypertens Res 2002; 25: 901–910)

Key Words: angiotensin II, afferent arterioles, efferent arterioles, prostaglandins, nitric oxide

Introduction

The renin-angiotensin (ANG) system constitutes a pivotal determinant of renal hemodynamics not only under physiological conditions (1–3), but also in the pathological state—such as in cases of renal ischemia and renal injury (4, 5). Although a large number of studies have been conducted to clarify the role of ANG II in the control of renal hemodynamics (1–3, 6–10), the findings obtained from these reports have been divergent. In whole kidney hemodynamic studies, ANG II has been reported to elevate the filtration fraction (6, 7), suggesting efferent arteriolar constriction. Furthermore, in micropuncture studies and isolated perfused microvessels, ANG II markedly constricts post-glomerular vessels in the superficial nephrons (1, 8). In contrast, in hydronephrotic...
kidneys in vitro (9, 10) and in vivo (11), afferent and efferent arterioles from superficial nephrons respond in a similar manner. In the juxtamedullary arterioles, ANG II has been shown to elicit a similar magnitude of vasoconstriction in the afferent and efferent arterioles in juxtamedullary nephrons preparations (2, 3) and in an in vivo hydronephrotic kidney model (11). It is also of interest that, in the latter model, the juxtamedullary arterioles exhibited blunted responses to ANG II compared with those of the superficial arterioles (11). However, these apparently discrepant observations were obtained under different experimental conditions, which may have confounded the interpretation of renal arteriolar reactivity to ANG II. Nevertheless, there have been no investigations examining the superficial and juxtamedullary arteriolar responses to ANG II under the same experimental conditions with a relatively less invasive technique.

Within the kidney, multiple vasoactive substances are synthesized in situ, and serve to modulate the renal arteriolar tone. Nitric oxide (NO) (8, 12) and prostaglandins (PG) (13, 14) are important paracrine/autocrine for helping to maintain the renal hemodynamics to counter a variety of vasoactive stimuli. However, zonal heterogeneity exists in the contents of these substances within the kidney. Both NO and PG are distributed more abundantly in the medulla than in the cortex (15–17). The zonal difference in these vasoactive substances would thus be anticipated to affect renal microvascular responsiveness to a variety of vasoactive substances. However, this premise has not yet been examined in vivo.

In the present study, we attempted to characterize the action of ANG II on the afferent and efferent arterioles in both superficial and juxtamedullary nephrons under identical experimental settings. In addition, we evaluated the role of intrarenal NO and PG in modulating the ANG II-induced constriction of the renal arterioles. In order to clarify these important issues, we used a novel technique known as intravital needle-type CCD camera videomicroscopy (15, 18), which allowed direct in situ, in vivo visualization of a relatively intact renal microcirculation. Finally, we also evaluated the intrarenal PG and NO contents using a renal microdialysis technique (15, 16, 19).

**Methods**

**Renal Hemodynamic Studies**

**Measurements of Systemic and Renal Hemodynamics**

All experimental procedures were conducted according to the guidelines of the Animal Care Committee of Keio University. Forty-five adult male mongrel dogs (8–13 kg) were fed a standard diet (Oriental Yeast Co., Tokyo, Japan) containing 65 mmol Na and 50 mmol K daily, and were anesthetized with pentobarbital (30 mg/kg). Free access to tap water was permitted at all times. After intratracheal intubation, each animal was ventilated with an artificial respirator and placed on a heating blanket to maintain body temperature at 37°C. A 7-Fr catheter (Create Medic, Tokyo, Japan) was inserted through the right femoral artery to measure mean arterial pressure (MAP) and heart rate (HR), and the left radial vein was catheterized for infusion of drug. A 7-Fr catheter was placed in the bladder for urine collection.

Through a retroperitoneal incision, an electromagnetic flow probe was placed around the renal artery for measurement of renal blood flow (RBF). Data of MAP, HR and RBF were analyzed with a Macintosh Laboratory system (Mac Lab; Analog-Digital Instruments, Castle Hill, Australia) (15, 20, 21). The glomerular filtration rate (GFR) was estimated from inulin clearance. Renal plasma flow (RPF) was calculated from RBF and hematocrit.

**Intravital CCD Camera Technique**

The methods used for evaluation of renal microcirculatory responses were detailed in our previous publications (15, 18). In brief, after the surgical procedure and instrumenta- tion, an intravital CCD camera probe was introduced into the midcortical layers of the left kidney. The probe was swung gently to obtain clear images of the superficial and jux- tamedullary nephrons. Superficial nephrons were observed with an upward view, while juxtamedullary nephrons were visible with a downward view. Afferent and efferent arterioles were distinguished on the basis of the direction of blood flow (15, 18).

**Measurements of Vessel Diameter**

Vessel diameters were measured as detailed previously (15, 18). Briefly, sequential images of renal microvessels were captured with a freeze-frame modality, and the density in the gray scale mode was digitized along the scanning line across the vessel. The difference between the peak and noise level was divided into quarters, and the position with the density of a quarter higher value above the noise level was identified as an inner diameter. A vessel segment approximately 15 μm in length was scanned, and the mean vessel diameter was det- ermined by averaging at least five measurements during the plateau of the response. The spatial resolution of this CCD system is 0.87 μm.

**Evaluation of Renal Interstitial Concentration of Prostaglandin E2 (PGE2) and NO**

Renal interstitial PGE2 and nitrite and nitrate (NOx) levels were assessed by a microdialysis technique (15, 16, 19). A microdialysis tube (0.5 mm diameter and a 10-kD transmembrane diffusion cutoff; Eicom, Kyoto, Japan) was insert- ed into the superficial cortex and outer medulla at a depth of 2 mm and 10 mm, respectively, from the renal surface. The position of the microdialysis tube was confirmed by echo- graphy. The microdialysis tube was perfused with lactated Ringer’s solution (147 mEq/l Na, 4 mEq/l K, 5 mEq/l Ca, 156 mEq/l Cl) at a rate of 2 μl/min. At this rate, in vitro recovery was 72% for PGE2, 78% for nitrite, and 70% for nitrate. A 180-min stabilization period was al-
lowed before initiation of the protocol. The effluent was collected at -20°C for PGE2 and nitrite/nitrate measurements. PGE2 was measured by RIA. NOx were evaluated with the Griess reaction (19), and the sum of these constituents was considered as a marker of renal NO levels (15).

**Experimental Protocols**

**Protocol 1: Renal Microvascular Effects of ANG II**

Sixty minutes were allowed for stabilization of renal hemodynamics. Thereafter, ANG II (Sigma, St. Louis, USA) was infused intravenously at increasing rates of 1, 3, 10, and 30 ng/kg/min, and the renal hemodynamics and arteriolar responses to ANG II were evaluated. Thirty minutes were required for observation of the renal microcirculation at each dose of ANG II.

**Protocol 2: Measurements of Intrarenal PGE2 and NOx Contents**

The effects of ANG II on renal contents of PG and NOx were assessed. After a 180 min stabilization period, basal levels of these substances were evaluated for 30 min. Thereafter, increasing doses of ANG II (1, 3, 10, and 30 ng/kg/min) were infused intravenously for 30 min. At each dose of ANG II, the effluent from microdialysis tubes was collected for 15 min.

**Protocol 3: Role of PG and NO in ANG II-Constricted Renal Arterioles**

The role of PGE2 and NO in modulating the ANG II-induced constriction of renal microvessels was assessed. Initially, either indomethacin (1 mg/kg; Sigma) or nitro-L-arginine methylester (L-NAME; 1 mg/kg; Sigma) was administered to block the activity of PG and NO. After 30 min of the drug administration, increasing doses of ANG II (i.e., 1, 3, 10, and 30 ng/kg/min) were infused, and the renal arteriolar responses to ANG II were compared with those in the absence of indomethacin and L-NAME.

Next, whether the NO-induced activities differed between the afferent and efferent arterioles was assessed by examining the effect of L-arginine on arteriolar tone. Following the injection of L-arginine (150 mg/kg), ANG II (1–30 ng/kg/min) was administered and its effect on arteriolar tone was evaluated.

**Statistics**

Data are expressed as the means ± SEM. Results were analyzed by 2-way analysis of variance (ANOVA), followed by Newman-Keuls’ post hoc test. Values of *p* < 0.05 were considered to indicate statistical significance.

**Results**

**Protocol 1: Renal Microvascular Effects of ANG II**

The administration of 10 ng/kg/min ANG II elevated MAP (n = 17), and a further elevation was observed at 30 ng/kg/min (Table 1). Heart rates were unaltered at any doses of ANG II. In contrast, RPF was decreased in response to 3 ng/kg/min ANG II, and showed a 36 ± 4% reduction at 30 ng/kg/min ANG II. GFR was reduced at the highest dose of ANG II (i.e., 30 ng/kg/min). Consequently, the filtration fraction was elevated by 3 ng/kg/min (*p* < 0.05) and 10 ng/kg/min ANG II (*p* < 0.05).
Fig. 2. Effects of angiotensin II on intrarenal levels of prostaglandin E2 (PGE2) and nitrite/nitrate (NOx) in canine kidneys. Angiotensin II produced dose-dependent elevations in PGE2 contents in both the cortex (open bars) and medulla (filled bars), although the medullary levels were greater at all doses (left). Medullary NOx contents were increased in response to angiotensin II, whereas a modest increase was observed in the cortex (right). Results are the means ± SEM. * p < 0.05 vs. baseline. † p < 0.05.

Table 1. Effects of Angiotensin II Administration on Renal Hemodynamics in Dog Kidneys

<table>
<thead>
<tr>
<th></th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
<th>RPF (ml/min)</th>
<th>GFR (ml/min)</th>
<th>FF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (n = 17)</td>
<td>110 ± 2</td>
<td>109 ± 2</td>
<td>153 ± 2</td>
<td>35 ± 2</td>
<td>0.21 ± 0.01</td>
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<tr>
<td>Saline</td>
<td>110 ± 3</td>
<td>109 ± 3</td>
<td>153 ± 3</td>
<td>35 ± 2</td>
<td>0.21 ± 0.01</td>
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<tr>
<td>Angiotensin II</td>
<td></td>
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<tr>
<td>1 ng/min/kg</td>
<td>110 ± 2</td>
<td>108 ± 2</td>
<td>147 ± 4</td>
<td>31 ± 2</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>3 ng/min/kg</td>
<td>114 ± 2</td>
<td>109 ± 2</td>
<td>133 ± 5**</td>
<td>31 ± 3</td>
<td>0.26 ± 0.02 *</td>
</tr>
<tr>
<td>10 ng/min/kg</td>
<td>121 ± 2*</td>
<td>110 ± 2</td>
<td>117 ± 5**</td>
<td>29 ± 2</td>
<td>0.24 ± 0.01 *</td>
</tr>
<tr>
<td>30 ng/min/kg</td>
<td>136 ± 3**</td>
<td>110 ± 3</td>
<td>97 ± 5**</td>
<td>21 ± 2**</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>Baseline (n = 16)</td>
<td>108 ± 2</td>
<td>105 ± 5</td>
<td>147 ± 2</td>
<td>35 ± 2</td>
<td>0.24 ± 0.01</td>
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<td>Indomethacin</td>
<td>109 ± 3</td>
<td>105 ± 5</td>
<td>138 ± 4</td>
<td>33 ± 2</td>
<td>0.24 ± 0.01</td>
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<tr>
<td>Angiotensin II</td>
<td></td>
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<td>1 ng/min/kg</td>
<td>110 ± 3</td>
<td>102 ± 5</td>
<td>131 ± 2</td>
<td>29 ± 3</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>3 ng/min/kg</td>
<td>113 ± 3</td>
<td>97 ± 5</td>
<td>116 ± 3*</td>
<td>30 ± 5</td>
<td>0.28 ± 0.04</td>
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<tr>
<td>10 ng/min/kg</td>
<td>127 ± 6*</td>
<td>104 ± 5</td>
<td>101 ± 4**</td>
<td>27 ± 6</td>
<td>0.31 ± 0.04 *</td>
</tr>
<tr>
<td>30 ng/min/kg</td>
<td>143 ± 5**</td>
<td>117 ± 5</td>
<td>84 ± 5**</td>
<td>21 ± 2*</td>
<td>0.31 ± 0.03 *</td>
</tr>
<tr>
<td>Baseline (n = 12)</td>
<td>109 ± 2</td>
<td>114 ± 2</td>
<td>148 ± 2</td>
<td>32 ± 1</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>L-NAME</td>
<td>118 ± 2*</td>
<td>99 ± 5*</td>
<td>123 ± 4*</td>
<td>31 ± 1</td>
<td>0.24 ± 0.01</td>
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<tr>
<td>Angiotensin II</td>
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</tr>
<tr>
<td>1 ng/min/kg</td>
<td>121 ± 2</td>
<td>100 ± 5</td>
<td>107 ± 4*</td>
<td>27 ± 1</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>3 ng/min/kg</td>
<td>122 ± 2</td>
<td>101 ± 6</td>
<td>100 ± 5*</td>
<td>23 ± 3*</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>10 ng/min/kg</td>
<td>130 ± 2*</td>
<td>103 ± 7</td>
<td>89 ± 5**</td>
<td>19 ± 1**</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>30 ng/min/kg</td>
<td>138 ± 3**</td>
<td>108 ± 6</td>
<td>80 ± 5**</td>
<td>18 ± 1**</td>
<td>0.23 ± 0.01</td>
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</table>

MAP, mean arterial pressure; HR, heart rate; RPF, renal plasma flow; GFR, glomerular filtration rate; FF, filtration fraction. ‡ p < 0.05; †† p < 0.01 vs. baseline. * p < 0.05; ** p < 0.01 vs. saline, indomethacin, L-NAME, or L-arginine in each group.

Figure 1 summarizes the effect of ANG II on renal arteriolar diameters in the superficial and juxtamedullary nephrons. In superficial nephrons, the afferent arterioles manifested dose-dependent constriction, with a 10 ± 3% decrease in diameter at 1 ng/kg/min (from 17.2 ± 0.7 to 15.6 ± 0.9 μm, p < 0.05, n = 15). At 30 ng/kg/min, ANG II elicited 46 ± 5%
to 8.7 ± 0.9 µm, p < 0.01) decrements in the diameter of afferent arterioles. The basal efferent arteriolar diameter (15.8 ± 0.5 µm, n = 15) tended to be smaller than that of the afferent arterioles (p = 0.1). Similar to its effect on the afferent arterioles, 1 ng/kg/min ANG II caused a significant constriction of the efferent arterioles (to 13.2 ± 0.6 µm, p = 0.01, n = 15), corresponding to a 14 ± 4% decrement in diameter (p > 0.05 vs. afferent arterioles). At 30 ng/kg/min, 53 ± 3% (p < 0.01) constriction responses were obtained (p > 0.05 vs. efferent arterioles).

In juxtamedullary nephrons, basal diameters of the afferent (21.1 ± 0.7 µm, n = 17) and efferent arterioles (19.5 ± 0.5 µm, n = 17) were greater than those of the respective arterioles of superficial nephrons (p < 0.01). In the efferent arterioles, ANG II elicited a 11 ± 4% vasoconstriction at 1 ng/kg/min (p < 0.01). Further addition of ANG II caused a dose-dependent constriction of this vessel, with 52 ± 3% decrements in diameter at 30 ng/kg/min (p < 0.01). In contrast, the afferent arterioles failed to show vasoconstrictor responses to 1 ng/kg/min (1 ± 3% decrements, p < 0.05 vs. 11 ± 4% decrements for efferent arterioles) or 3 ng/kg/min ANG II (6 ± 3% decrements, p < 0.01 vs. 25 ± 3% decrements for efferent arterioles). A significant constriction was observed at 10 ng/kg/min (17.9 ± 0.9 µm, p < 0.05); this response (17 ± 4% decrement in diameter) was markedly diminished compared with that of the efferent arterioles (37 ± 4% decrements, p < 0.01). At 30 ng/kg/min, the diameter of the afferent arterioles decreased by 44 ± 4% (p = 0.1 vs. efferent arterioles).

**Protocol 2: Measurements of Intrarenal PGE2 and NOx Contents**

The administration of ANG II tended to enhance intrarenal PGE2 production in the superficial cortex (Fig. 2); at 30 ng/kg/min, the PGE2 contents increased from 305 ± 29 to 879 ± 177 pg/ml (p < 0.05, n = 8). In the medulla, the basal levels of intrarenal PGE2 contents were 2-fold greater (635 ± 31 pg/ml, n = 7) than in the cortex (p < 0.05). ANG II elicited more marked increases in intrarenal PGE2 contents, with the contents of medullary PGE2 being greater than the cortical levels at all doses of ANG II (p < 0.05). Thus, the PGE2 level was significantly elevated by 1 ng/kg/min ANG II (from 635 ± 31 to 850 ± 100 pg/ml, p < 0.05), and 2-fold increases were observed at 30 ng/kg/min (1,703 ± 206 pg/ml, p < 0.01).

ANG II had modest effect on cortical NOx contents (Fig. 2); at 30 ng/kg/min, the NOx level increased from 6.5 ± 0.3 to 8.3 ± 0.4 µmol/l (p < 0.05, n = 12). In the medulla, the basal NOx level (9.0 ± 0.5 µmol/l, n = 13) was higher than in the cortex (p < 0.05). A significant increase was seen at 10 ng/kg/min ANG II (p < 0.05); at 30 ng/kg/min, the
medullary NO contents reached 12.2 ± 1.1 µmol/l (p < 0.05).

Protocol 3: Effect of NO and PG on ANG II-Constricted Renal Arterioles

Indomethacin had no effect on the systemic (MAP and HR) or renal hemodynamics (RPF, GFR, and filtration fraction) (Table 1). The administration of indomethacin did not alter the arteriolar diameter of superficial nephrons (Fig. 3). In juxtamedullary nephrons, indomethacin tended to decrease the afferent arteriolar diameter (p = 0.08, n = 21), but had no effect on the efferent arteriolar diameter (p > 0.2, n = 22).

Indomethacin did not alter the ANG II-induced constriction of afferent (n = 10) or efferent arterioles of superficial nephrons (n = 10; Fig. 4, squares): the responsiveness with and without indomethacin were nearly the same (p > 0.5; circles). Similarly, indomethacin had no effect on the ANG II-induced constriction of the juxtamedullary efferent arterioles (p > 0.5, n = 22). In contrast, indomethacin augmented the ANG II-induced constriction of the juxtamedullary afferent arterioles; at 1, 3 and 10 ng/kg/min, 11 ± 3%, 19 ± 3%, and 35 ± 2% (n = 21) vasoconstrictor responses were observed (p < 0.05, p < 0.01, p < 0.01 vs. in the absence of indomethacin), respectively. Consequently, in juxtamedullary nephrons, indomethacin abolished the difference between afferent and efferent arteriolar responsiveness to ANG II (p > 0.1).

The blockade of NO synthesis by L-NAME elevated MAP (p < 0.01) and decreased RPF (p < 0.01; Table 1). In this setting, ANG II significantly elevated MAP at 10 ng/kg/min, and reduced RPF at 1 ng/kg/min. GFR was decreased at 3 ng/kg/min, and showed a 35 ± 4% reduction at a dose of 30 ng/kg/min ANG II.

The administration of L-NAME induced marked constriction of the renal arterioles in both superficial (p < 0.01, n = 11) and juxtamedullary nephrons (p < 0.01, n = 13) (Fig. 3). In the presence of L-NAME, superficial afferent and efferent arteriolar responses to 1 ng/kg/min ANG II were enhanced (Fig. 4, triangles) compared with those in the absence of L-NAME (circles, p < 0.05). Further increases in ANG II ad-
ministration (3–30 ng/kg/min), however, elicited nearly the same magnitude of constriction of the afferent and efferent arterioles as seen in the absence of L-NAME (p > 0.5). In the juxtamedullary nephrons, both arterioles exhibited greater constriction than in the absence of L-NAME; increasing doses (1, 3, and 10 ng/kg/min) of ANG II caused 16% - 3% (p < 0.05), 23% - 4% (p < 0.01), and 29% - 5% constriction of afferent arterioles (p < 0.05), respectively. Similarly, efferent arteriolar responses to ANG II were enhanced, with 27% - 4% and 39% - 4% decrements in diameter at 1 and 3 ng/kg/min, respectively (p < 0.05 vs. in the absence of L-NAME). It is noteworthy that, in the presence of L-NAME, the superficial afferent and efferent arterioles showed similar constrictor responses to ANG II, whereas in juxtamedullary nephrons, the afferent arteriolar responses to ANG II were less than those of efferent arterioles (p < 0.05, p < 0.01, p < 0.01 for 1, 3, and 10 ng/kg/min ANG II, respectively; Fig. 4, triangles).

The infusion of L-arginine did not alter MAP (from 111 ± 7 to 111 ± 10 mmHg, n = 8), but increased the diameters of superficial arterioles and tended to dilate the juxtamedullary arterioles (Fig. 3). Subsequently, increasing doses of ANG II failed to elevate MAP, with only a modest increase in MAP (to 126 ± 8 mmHg) observed at 30 ng/min/kg. Furthermore, renal arteriolar responses to ANG II were markedly blunted compared with those in the absence of L-arginine; a dose of 30 ng/min/kg ANG II was required to elicit a significant constriction of the afferent and efferent arterioles in superficial and juxtamedullary nephrons (Fig. 4, inverted triangles). Of note, the constrictor responses of the afferent arterioles were less than those of the efferent arterioles in both superficial (at 10 and 30 ng/kg/min) and juxtamedullary nephrons (at 30 ng/kg/min; Fig. 4).

**Discussion**

In the present study, ANG II produced marked constriction of the renal arterioles, as revealed using the novel technique of intravital needle-type CCD videomicroscopy. Thus, in superficial nephrons, ANG II produced dose-dependent constriction, with similar constrictor responses observed in afferent and efferent arterioles. In contrast, in juxtamedullary nephrons the renal arterioles manifested differing responsiveness; the afferent arterioles required a higher dose of ANG II (10 ng/kg/min) to obtain a constrictor response than the efferent arterioles (1 ng/kg/min). These findings therefore support the formulation that the kidney expresses segmental (afferent vs. efferent) heterogeneity in its microvascular reactivity to ANG II (22, 23). Furthermore, the different responsiveness of the superficial and juxtamedullary arterioles is consistent with a previous study in which the effects of ANG II on regional blood flow exhibited zonal heterogeneity, i.e., the ANG II-induced decrease in blood flow in the outer cortex was greater than the decrease in inner medullary flow, as revealed by Laser Doppler (24).

Previous studies have reported divergent results regarding the effects of ANG II on renal microvessels. In perfused microvessels isolated from the superficial cortex, Ito et al. (25) demonstrated that although ANG II produced a similar magnitude of constriction of the afferent and efferent arterioles at the maximal dose, the efferent arterioles were more sensitive to ANG II than the afferent arterioles. In contrast, in the in vivo and in vitro hydronephrotic kidney models, the renal arterioles of superficial nephrons exhibited similar constrictor responses to ANG II in the afferent and efferent arterioles (10, 26). In juxtamedullary nephrons, Carmines et al. (2) and Steinhausen et al. (11) demonstrated nearly identical constriction of afferent and efferent arterioles. Of note, Steinhausen reported that both arterioles of juxtamedullary nephrons manifested diminished constrictor responses to ANG II, compared with those of superficial nephrons, and suggested a zonal difference in the renal microvascular response to ANG II. Although the above findings appear discrepant, they were obtained in different experimental settings or by means of a model in which the perivascular circumstances might have been altered. Since our new technique allows direct visualization of the in vivo renal microcirculation under the same experimental setting and under less invasive conditions (15, 18), our present findings indicate zonal (juxtamedullary vs. superficial nephrons) and segmental (afferent vs. efferent arterioles) heterogeneity in the in vivo, in situ renal microvascular action of ANG II.

The discrepant findings in regard to the responsiveness of the renal microvessels to ANG II may be related to the heterogeneous distribution of intrarenal paracines/autoclines. It has been well established that the kidney is enriched with a large amount of PG, with zonal heterogeneity in its content between cortex and medulla (17). Indeed, the present study showed that the contents of PGE2 were greater in the medulla than in the cortex, and this profile of intrarenal PGE2 contents was preserved during the ANG II administration (Fig. 2). The greater PG level in the medulla is anticipated to modify the responsiveness of the neighboring juxtamedullary arterioles more greatly than that of superficial vessels. Thus, the inhibition of PG synthesis markedly enhanced the juxtamedullary afferent arteriolar responsiveness to ANG II, whereas no augmentation was observed in the superficial afferent arterioles (Fig. 4, squares). Consequently, the PG synthesis inhibition abolished the zonal (i.e., superficial vs. juxtamedullary) heterogeneity in the afferent arteriolar reactivity to ANG II. These observations indicate an active contribution of intrarenal PG to the ANG II-mediated vascular tone of afferent arterioles in the juxtamedullary, but not the superficial, cortex. In this regard, Harrison-Bernard and Carmines (27) demonstrated that cyclooxygenase inhibition by piroxicam enhanced the juxtamedullary afferent arteriolar responses to ANG II, and suggested that the afferent arteriolar responses to ANG II were modulated by cyclooxygenase products in juxtamedullary nephrons. Steinhausen et al. (11) also reported an augmented ANG II-induced jux-
tamedullary afferent arteriolar constriction by indomethacin, using the in vivo hydronephrotic kidney model. Alternatively, the vasodilator response to PGE2 at the EP4 receptor level or the activity of other arachidonate substances (28) might differ quantitatively between the superficial and juxtamedullary afferent arterioles.

It is worth noting that the dose of the cyclooxygenase inhibitor used in the current study had no effect on the basal hemodynamics or arteriolar diameters (Table 1 and Fig. 3), although indomethacin tended to reduce the juxtamedullary afferent arterioles, an observation in parallel with that reported by Harrison-Bernard and Carmines (27). When stimulated by ANG II, however, the PG production appears to buffer the vasoconstriction of juxtamedullary afferent arterioles, although the subsequent change in glomerular hemodynamics in juxtamedullary nephrons affects renal hemodynamics negligibly. Nevertheless, PG is reported to serve to maintain the renal hemodynamics when the kidney is exposed to compromised conditions (4, 5). Thus, the ANG II-stimulated—but not the basal—PG production constitutes an important determinant of the zonal heterogeneity of renal arteriolar responsiveness to ANG II. Whether this formulation is relevant to pathological conditions such as renal artery stenosis (29) requires further investigations.

In contrast to the afferent arteriolar response, the cyclooxygenase inhibition failed to alter the efferent arteriolar responsiveness to ANG II. This finding is in good agreement with the observations by Tang et al. (30), who reported that PGE2 had no effect on the reactivity of the efferent arterioles to ANG II. Similarly, in the juxtamedullary nephron preparation, piroxycam is reported to have no effect on efferent arteriolar response to ANG II (27). Thus, the lack of effect of PGE2 on the efferent arterioles would explain the non-modulation by indomethacin of ANG II-induced constriction of this vessel. Alternatively, the preferential modulation by PG of the ANG II-induced responsiveness at the juxtamedullary afferent arterioles would be accounted for by the segmental heterogeneity in arteriolar responsiveness (i.e., the failure of the efferent arterioles to respond to PGE2), in addition to the zonal heterogeneity in intrarenal PG distribution (i.e., the preferential activity of PGE2 in the juxtamedullary region).

The present study indicates that intrarenal NO exerts pronounced activity in mediating the renal microvascular tone under basal and ANG II-stimulated conditions. Thus, the infusion of L-NAME decreased the basal afferent and efferent arteriolar diameters in both superficial and juxtamedullary nephrons (Fig. 3). Furthermore, the vasoconstrictor responsiveness to ANG II was enhanced in all arterioles examined (Fig. 4). These observations suggest that NO plays an important role in modulating the renal arteriolar tone in both juxtamedullary and superficial nephrons. Of interest, the L-NAME-induced augmentation of arteriolar responses was greater in juxtamedullary nephrons than in superficial nephrons (Fig. 4; note the differences between circles and triangles in each dose of ANG II). Thus, NO is expected to make a greater contribution to the arteriolar responses to ANG II in juxtamedullary nephrons than in superficial nephrons. Indeed, the present study further shows that the medullary NO content is greater than the cortical content at all doses of ANG II (Fig. 2), a finding in agreement with that reported by Zou et al. (31). Together, these results indicate that the role of NO in renal microvascular tone differs depending on the zonal (i.e., medulla vs. cortex) localization of the arterioles.

Of importance is the finding that, in the presence of L-NAME, the ANG II-induced afferent arteriolar constriction was less than that of efferent arterioles in juxtamedullary nephrons (Fig. 4, triangles), indicating that the diminished contractile property of juxtamedullary afferent arterioles was preserved during NO inhibition. In this regard, Ito et al. (25) demonstrated in perfused microvessels isolated from the rabbit superficial cortex that afferent arterioles were more resistant to the vasoconstrictor action of ANG II than efferent arterioles, and further indicated that this differential responsiveness was abolished by L-NAME. In contrast, Ohishi et al. (32) reported that NO inhibition did not alter the ANG II-induced constriction of afferent or efferent arterioles in the juxtamedullary nephron preparation. Although the reason for these divergent findings observed during the modulation of renal NO production remains unclear, a different degree of renal NO modulation and sensitivity to NO, as well as differences in the experimental settings, may have affected the arteriolar reactivity to ANG II. Indeed, we also have observed that L-arginine blunts the ANG II-induced constriction more markedly in the afferent than the efferent arterioles (superficial, at 10 and 30 ng/kg/min; juxtamedullary, at 30 ng/kg/min; Fig. 4, inverted triangles). Despite these divergent observations, the present study suggests that intrarenal NO activity is not a major determinant of the segmental (i.e., afferent vs. efferent) difference in arteriolar responsiveness to ANG II in the juxtamedullary nephrons.

Finally, the effect of systemic blood pressure on the ANG II-induced arteriolar constriction merits comment. In the present study, we administered ANG II into the systemic circulation (i.e., the femoral vein), which would elevate MAP and possibly enhance the pressure-induced (e.g., myogenic) constriction observed at the afferent, but not the efferent, arterioles (33). It was previously demonstrated that juxtamedullary afferent arterioles manifested less pressure-induced constriction than superficial arterioles (11). The difference in the pressure-induced constriction may therefore contribute to the diminished responsiveness of the juxtamedullary vs. the superficial arterioles. This possibility seems unlikely, however, since distinct afferent arteriolar responses were observed at relatively lower doses of ANG II (1–3 ng/min/kg) despite the lack of change in MAP. Furthermore, the pretreatment with indomethacin did not significantly augment the hypertensive actions of ANG II in our study (Table 1). Finally, although L-NAME elevated MAP, the augmentation by L-NAME of ANG II-induced afferent...
arteriolar constriction was rather greater in the juxtamedullary than in the superficial nephrons (Fig. 4). In contrast, these observations favor our premise that the heterogeneity in afferent arteriolar responses to ANG II in the superficial and juxtamedullary nephrons is independent of the effect of elevated systemic blood pressure.

In conclusion, our new technique to directly visualize the renal microcirculation in vivo, i.e., needle-type CCD videomicroscopy, has elucidated the zonal (juxtamedullary vs. superficial nephrons) and segmental (afferent vs. efferent arterioles) heterogeneity in the renal microvascular action of ANG II in the same experimental setting. Such heterogeneity is most likely associated with the difference in the intrarenal production of PGE2 and NO under basal and during ANG II-stimulated conditions. The role of PGE2 and NO in mediating the arteriolar responsiveness, however, also differs, with PGE2 contributing to segmental and zonal differences in arteriolar responses. In contrast, NO is responsible mainly for the zonal heterogeneity in arteriolar responsiveness. These two vasoactive substances would thus serve in concert to modulate the renal microvascular tone in vivo.

References


