Effect of Exogenous Angiotensin II on Renal Hemodynamics in the Awake Rat

Measurement of Afferent Arteriolar Diameter by the Microsphere Method

CHEN H. HSU, THEODORE W. KURTZ, AND JAMES M. SLAVICEK

SUMMARY We measured cardiac output (CO), renal blood flow (RBF), renal plasma flow (RPF), and afferent arteriolar diameter by the microsphere method, and inulin clearance (GFR) simultaneously in awake rats given an infusion of exogenous angiotensin II (100 ng/min per kg). Angiotensin II did not affect the CO, whereas both RBF and RPF decreased significantly in rats infused with angiotensin II (RBF, 2.88 ± 0.11, mean ± SEM; RPF, 1.78 ± 0.09, n = 6) when compared to control animals given saline (RBF, 4.58 ± 0.31; RPF, 2.80 ± 0.24 ml/min per 100 g, n = 6, both P < 0.005, respectively). Both mean arterial pressure (MAP) and renal vascular resistance (RVR) were significantly higher in rats infused with angiotensin II than in controls. The decrease in GFR did not parallel the reduction of RPF in rats infused with angiotensin II as reflected by their higher values of mean filtration fraction (41.1 ± 1.6%, n = 6) than that of controls (33.7 ± 2.4%, n = 6, P < 0.05). Despite significant elevations of MAP and RVR in rats infused with angiotensin II, their mean afferent arteriolar diameter (19.6 ± 0.24 μm) was not different from that of controls (20.1 ± 0.39 μm). We conclude that angiotensin II preferentially acts at the site of postglomerular vasculature but not at the afferent arteriole. Circ Res 46: 646–650, 1980.
recently developed microsphere technique (Chenitz et al., 1976; Ofstad et al., 1975) to measure directly afferent arteriolar diameter in rats infused with angiotensin II.

Methods

Studies were performed on 12 Sprague-Dawley rats weighing between 250 and 290 g. The animals were fed with Purina rat chow and tap water ad libitum. They were weighed and lightly anesthetized with ether, and polyethylene cannulae (PE 10) were placed in the femoral artery and through the carotid artery into the left ventricle. The femoral vein also was cannulated with polyethylene tubing (PE 50) for infusion. A PE 50 tube for urine collection was inserted into the urinary bladder through a small suprapubic abdominal incision. After surgery, the rats were placed in restraining cages and allowed to recover for at least 1 hour prior to the experiment. The animals were divided into two groups for the following studies.

After baseline arterial pressure had been measured through the cannula in the femoral artery, six rats in the experimental group were primed with inulin, 20 mg/100 g body weight, followed by a sustaining infusion of inulin in isotonic saline solution containing angiotensin II (Ciba Pharmaceutical) at a rate of 0.05 ml/min. This rate of infusion delivered approximately 100 ng/min per kg of angiotensin II. Six control rats were given an infusion of inulin in isotonic saline solution without angiotensin II at a rate of 0.05 ml/min. Mean arterial pressure was monitored using a Statham model P23Db strain gauge with a Gilson recorder throughout the experiment. The arterial pressure usually increased within 15 minutes following angiotensin II infusion and stabilized thereafter. Urine was collected for 30-45 minutes after the sustaining infusion, and 1.0 ml of blood was withdrawn at the midpoint of the urine collection for determination of inulin clearance.

At the end of the clearance period, cardiac output (CO), renal blood flow (RBF), and mean afferent arteriolar diameter were measured simultaneously in each rat by microspheres as previously described (Hsu et al., 1977). Radioactive microspheres labeled with $^{85}$Sr (14.5 ± 2 μm) were mixed with nonlabeled microspheres (22.3 ± 3 μm) (3M Company) in a proportion of 1:2 with a final concentration of 3 mg/ml. Approximately 0.15 ml of microspheres in 10% dextran solution was injected into the left ventricle through the carotid catheter within 10 seconds. A hypotensive effect was not observed following the dextran and microsphere injection. Before the syringe was filled, the solution containing microspheres was agitated vigorously with a Vortex mixer for at least 5 minutes. After the syringe had been filled to a volume of 0.15 ml, it was capped with a hub, and radioactivity was counted for 30 seconds in a Packard γ counter. Immediately after injection of the microspheres into the left ventricle, approximately 0.1-0.2 ml of blood was collected through the femoral arterial catheter by free flow into a preweighed tube for exactly 1 minute as a reference blood sample.

After the femoral blood collection, 0.1 ml of blood was withdrawn from the carotid catheter to clear it of residual isotope. The blood withdrawn from the carotid catheter then was counted along with the syringe, hub, and catheter needle, to determine residual isotope counts. This value was subtracted from the preinjection counts to yield total counts injected. Approximately 60,000 microspheres were administered. Immediately after the isotope injection, rats were lightly anesthetized with intravenous pentobarbital (20 mg/kg) and both kidneys were removed, decapsulated, and counted for radioactivity. Blood samples collected after the injection of microspheres also were counted and weighed. The blood volume was calculated by dividing the weight of the blood sample by the specific gravity of rat blood, which previously had been determined to be 1.063 g/ml ($n = 8$). RBF of both kidneys combined was calculated as: $RBF = \frac{\text{two kidneys (counts/min)}}{\text{femoral blood (counts/min)}} \times \text{femoral blood flow rate (ml/min per 100 g body weight)}$. Renal vascular resistance (RVR) was calculated as: $RVR = \frac{\text{MAP}}{\text{RBF}}$. Renal plasma flow (RPF) was calculated as: $\text{RPF} = \text{RBF} \times (1 - \text{Hct})$. CO was calculated as: $\text{CO} = \frac{Q}{\text{femoral blood (counts/min)}} \times \text{femoral blood flow rate (ml/min per 100 g body weight)}$ where Q is the total amount of microspheres (counts/min) injected.

Afferent arteriolar diameter was assessed with the microsphere method previously described (Chenitz et al., 1976; Ofstad et al., 1975). Basically, any microsphere with a diameter smaller than that of an afferent arteriole will pass through the vessel and be trapped in a glomerulus. Conversely, if the sphere is larger than the arteriole, it will lodge outside the glomerulus. Thus, the frequency distribution of the various sizes of microspheres trapped within the afferent arterioles can be used to estimate afferent arteriolar diameter.

After isotopic counting, the kidneys were exposed serially to increasing concentrations of ethanol, cleared with methyl salicylate, then embedded in Paraplast (Sherwood Medical, Inc.) before being sliced with a microtome (American Optical 900). Twelve to 20 sagittal sections (120-μm thickness), consisting of cortex and medulla, were prepared from each kidney. Each tissue slice was soaked in xylene to remove the Paraplast, hydrated, and stained by immersion in 2% alcian blue for 15 minutes. The stained tissue slices were dehydrated again as described above and mounted in balsam (Supermount, Paragon C & C, Inc.) for light microscopic examination at 400x. The vasculature and glomeruli could be identified readily. Approximately one microsphere was found within a glo-
merulus or its afferent arteriole per 8 to 10 glomeruli examined.

A micrometer eyepiece with an accuracy of 0.2 μm (American Optical) was used to measure the range of diameters of the injected microspheres. Their range is depicted in Figure 1. In addition, the diameters of at least 130 microspheres (range 138-270) trapped within clearly identifiable afferent arterioles were measured for each rat. Microspheres trapped at the junctions of afferent arterioles with glomerular capillary loops (at a hilar position) were included, since the data of Morkrid et al. (1978) show that these spheres have diameters similar to those of spheres located more proximally in the afferent arteriole. With these data, a histogram was constructed that depicted the distribution of the diameters of the trapped microspheres for each rat (Fig. 2). Mean afferent arteriolar diameter was calculated by using these distributions of the diameters according to the formula developed by Ofstad et al. (1975). This method is based on a mathematical model which assumes that three main factors determine the diameter distribution of microspheres trapped within afferent arterioles in the kidney. These factors are (1) the diameter distribution of the injected microsphere population, (2) the diameter distribution of the afferent arterioles, and (3) the afferent arteriolar blood flow which is proportional to the fourth power of the afferent arteriolar diameter. The range of the diameters of the injected microspheres and the distribution diameters of trapped microspheres are experimentally determined. Because afferent arteriolar blood flow is proportional to the fourth power of the diameter of the microsphere trapped within the vessel, one can derive the range of diameters of the afferent arterioles and, hence, mean afferent arteriolar diameter. The mathematical details of this approach have been outlined clearly by Ofstad et al. (1975). All statistical analyses were made by Student's t-test. Values are expressed as mean ± SEM.

Results

Mean values of CO, RBF, RPF, MAP, RVR, inulin clearance (GFR), filtration fraction (FF), and afferent arteriolar diameter in control and experimental rats are presented in Table 1. Angiotensin II did not affect the CO, whereas RBF and RPF both decreased significantly in rats infused with angiotensin II (RBF, 2.88 ± 0.11; RPF, 1.78 ± 0.09, n = 6) when compared with control rats given saline (RBF, 4.58 ± 0.31; RPF, 2.80 ± 0.24 ml/min per 100 g body weight, both P < 0.005). Both MAP and RVR were significantly higher in rats infused with angiotensin II than in controls. The decrease in GFR did not parallel the reduction of RBF in rats infused with angiotensin II, as reflected by their higher value for FF (41.1 ± 1.6%) than that of controls (33.7 ± 2.4%, P < 0.05). Despite significant elevations of MAP and RVR in rats infused with angiotensin II, their mean afferent arteriolar diameter was not different from that of control animals.

Discussion

The validity of the microsphere method for measurement of CO and RBF in the rat has been documented previously (Hsu et al., 1977; Mendell and Hollenberg, 1971; Sasaki and Wagner, 1971). Our values for CO and RBF in control rats are similar to those in these reports. Assessment of the afferent arteriolar diameter using the microsphere method was developed by Ofstad et al. (1975) and has been used in various investigations (Chenitz et al., 1976; Hsu et al., 1979; Morkrid et al., 1978). In the present study, we modified our previous methodology (Hsu et al., 1979) by increasing the range of diameter of the injected microsphere population (11-28 μm, Fig. 1). Consequently, the mean afferent arteriolar diameter of control rats in this study is slightly smaller than our previous result (20.1 ±
TABLE 1 Effect of Exogenous Angiotensin II on Systemic and Renal Hemodynamics, Inulin Clearance, and Afferent Arteriolar Diameter

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>co (ml/min per 100 g)</th>
<th>RBF (ml/min per 100 g)</th>
<th>RPF (ml/min per 100 g)</th>
<th>MAP (mm Hg)</th>
<th>Cm (ml/min per 100 g)</th>
<th>FF (%)</th>
<th>Afferent arteriolar diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2436</td>
<td>38.3</td>
<td>5.81</td>
<td>3.89</td>
<td>124</td>
<td>21.3</td>
<td>0.99</td>
<td>25.5</td>
</tr>
<tr>
<td>2446</td>
<td>29.9</td>
<td>3.75</td>
<td>2.18</td>
<td>114</td>
<td>30.4</td>
<td>0.91</td>
<td>41.7</td>
</tr>
<tr>
<td>2449</td>
<td>29.3</td>
<td>5.02</td>
<td>2.96</td>
<td>116</td>
<td>23.1</td>
<td>0.89</td>
<td>30.1</td>
</tr>
<tr>
<td>2450</td>
<td>23.9</td>
<td>4.98</td>
<td>2.45</td>
<td>108</td>
<td>26.5</td>
<td>0.96</td>
<td>39.2</td>
</tr>
<tr>
<td>2453</td>
<td>29.0</td>
<td>4.63</td>
<td>2.64</td>
<td>112</td>
<td>24.2</td>
<td>0.90</td>
<td>34.1</td>
</tr>
<tr>
<td>2456</td>
<td>25.2</td>
<td>4.17</td>
<td>2.67</td>
<td>124</td>
<td>29.7</td>
<td>0.85</td>
<td>31.8</td>
</tr>
</tbody>
</table>

Mean ± SEM 29.6 ± 1.9 4.58 ± 0.31 2.88 ± 0.24 116.3 ± 2.7 25.9 ± 1.5 0.92 ± 0.02 33.7 ± 2.4 20.1 ± 0.39

Control: Infusion of normal saline at a rate of 0.05 ml/min

Experiment: Infusion of angiotensin II, 100 ng/min per kg, at a rate of 0.05 ml/min

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>co (ml/min per 100 g)</th>
<th>RBF (ml/min per 100 g)</th>
<th>RPF (ml/min per 100 g)</th>
<th>MAP (mm Hg)</th>
<th>Cm (ml/min per 100 g)</th>
<th>FF (%)</th>
<th>Afferent arteriolar diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2438</td>
<td>38.2</td>
<td>3.26</td>
<td>1.96</td>
<td>154</td>
<td>47.2</td>
<td>0.78</td>
<td>39.8</td>
</tr>
<tr>
<td>2440</td>
<td>27.8</td>
<td>3.00</td>
<td>1.83</td>
<td>148</td>
<td>49.3</td>
<td>0.77</td>
<td>42.1</td>
</tr>
<tr>
<td>2442</td>
<td>26.6</td>
<td>2.97</td>
<td>1.78</td>
<td>132</td>
<td>44.4</td>
<td>0.66</td>
<td>37.1</td>
</tr>
<tr>
<td>2445</td>
<td>32.8</td>
<td>2.68</td>
<td>1.58</td>
<td>144</td>
<td>53.7</td>
<td>0.67</td>
<td>42.4</td>
</tr>
<tr>
<td>2447</td>
<td>23.9</td>
<td>2.49</td>
<td>1.36</td>
<td>140</td>
<td>56.2</td>
<td>0.65</td>
<td>47.8</td>
</tr>
<tr>
<td>2451</td>
<td>26.8</td>
<td>2.86</td>
<td>1.70</td>
<td>140</td>
<td>49.0</td>
<td>0.64</td>
<td>37.7</td>
</tr>
</tbody>
</table>

Mean ± SEM 29.4 ± 2.1 2.88 ± 0.11 1.78 ± 0.09 143 ± 3.1 50.0 ± 1.8 0.70 ± 0.03 41.1 ± 1.6 19.6 ± 0.24

P value >0.05 <0.005 <0.005 <0.005 <0.001 <0.001 <0.05 >0.05

Abbreviation: Cm, inulin clearance.

0.39 µm, n = 6, vs. 22.0 ± 0.16 µm, n = 6). This is due primarily to the increase in the range of diameters of injected microspheres to include a size smaller than 17 µm. As a result, the smaller arteries which were not detected previously are included in this study. The mean afferent arteriolar diameter of 20 µm in the rat is fairly close to the value recently obtained by Morkrid et al. (1978) in the dog. We believe that measurement of afferent arteriolar diameter using this microsphere method is a sensitive technique, as it has been shown to detect changes in diameter of the afferent arterioles in hemorhagic hypotension (Ofstad et al., 1973), following a decrease in renal arterial pressure by aortic clamp (Morkrid et al., 1978), and in acute renal failure induced by intramuscular injection of glycerol (Hsu et al., 1979).

The preponderance of recent evidence has indicated that the efferent vasculature is the primary site of action of angiotensin II. Davalos et al. (1978), in the isolated perfused rat kidney, demonstrated that if the perfusate contained angiotensin II, there was a decrease in the perfusate flow and an increase in glomerular capillary hydrostatic pressure and proximal tubular pressure. Therefore, they used micropuncture techniques to study the effects of angiotensin II on afferent and efferent vascular resistances. Their study indicated that angiotensin II exerts a greater effect on the efferent arteriole but also has an effect on the afferent arteriole. The method used to determine the afferent arteriolar resistance does not actually localize the resistance to the afferent arteriole. The calculation provides only for the estimation of pregglomerular vascular resistance which could be affected by changes in vessel diameter in any part of the preglomerular vascular bed including the renal artery. This is due to the fact that the equation used to calculate resistance employs the pressure drop from the systemic level (measured in the femoral artery) to the glomerular capillary. Kallskog et al. (1976) recently have shown that substantial changes in pregglomerular resistance can occur in the interlobular arteries, which further supports the contention that the afferent arteriole may not necessarily be the primary or sole site of regulation of pregglomerular resistance. Finally, also open to question is whether or not the changes in pre- and postglomerular re-

after reduction of renal arterial perfusion pressure. These observations further support the hypothesis that the renin-angiotensin system participates in the regulation of GFR through its predominant effect on the efferent glomerular vasculature.

The previous studies have employed indirect approaches involving measurements of GFR, RBF, and FF to localize the intrarenal site of action of angiotensin II. Myers et al. (1975), however, have indicated that such indirect approaches may not be entirely reliable without simultaneous knowledge of glomerular capillary hydrostatic pressure and proximal tubular pressure. Therefore, they used micropuncture techniques to study the effects of angiotensin II on afferent and efferent vascular resistances. Their study indicated that angiotensin II exerts a greater effect on the efferent arteriole but also has an effect on the afferent arteriole. The method used to determine the afferent arteriolar resistance does not actually localize the resistance to the afferent arteriole. The calculation provides only for the estimation of pregglomerular vascular resistance which could be affected by changes in vessel diameter in any part of the preglomerular vascular bed including the renal artery. This is due to the fact that the equation used to calculate resistance employs the pressure drop from the systemic level (measured in the femoral artery) to the glomerular capillary. Kallskog et al. (1976) recently have shown that substantial changes in pregglomerular resistance can occur in the interlobular arteries, which further supports the contention that the afferent arteriole may not necessarily be the primary or sole site of regulation of pregglomerular resistance. Finally, also open to question is whether or not the changes in pre- and postglomerular re-

EFFECT OF ANGIOTENSIN ON RENAL VASCULATURE/Hsu et al. 649
sistance observed after angiotensin II by Myers et al. (1975) truly reflect a pharmacological action of angiotensin II on the intrarenal vasculature. Although attempts to control renal perfusion pressure were made by aortic clamping during angiotensin II infusion, the elevated systemic arterial pressure and resistance could have decreased CO, thereby causing secondary changes in intrarenal vascular resistance not due to a specific effect of angiotensin II itself. The dosage of angiotensin II administered by Myers et al. (1975) ranged from 200 to 600 ng/min per kg, and our preliminary studies showed consistent decreases in CO to less than 60% of normal whenever 200 ng/min per kg were infused. Therefore, we used a pressor dose of angiotensin II of 100 ng/min per kg in this study and were able to increase the systemic and renal vascular resistances without affecting the CO.

The results of the present study suggest that angiotensin II causes a significant increase in renal vascular resistance without inducing afferent arteriolar vasoconstriction. Our findings of increased FF and decreased RBF also are consistent with the results from other laboratories, indicating a preferential effect of angiotensin II on the postglomerular vasculature. This study, however, cannot exclude the possible effect of angiotensin II on the intrarenal vasculature proximal to the afferent arteriole.

Acknowledgments

We are grateful to Laura K. Larisey for the preparation of this manuscript.

References


Effect of exogenous angiotensin II on renal hemodynamics in the awake rat. Measurement of afferent arteriolar diameter by the microsphere method.

C H Hsu, T W Kurtz and J M Slavicek

doi: 10.1161/01.RES.46.5.646

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1980 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/46/5/646

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ 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 _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/