Relation of Renal Hemodynamics to Metabolism of Angiotensin II by the Canine Kidney

MICHAEL D. BAILIE AND SUZANNE OPARIL

SUMMARY The effects of changes in glomerular filtration rate and renal blood flow on the renal handling of ¹⁴C- or ¹²⁵I-labeled angiotensin II were studied in pentobarbital-anesthetized dogs. Labeled angiotensin II was injected as a bolus directly into the renal artery and the renal venous effluent was collected. Under control conditions, 52 ± 8% of the labeled material recovered was angiotensin II and the remaining 48 ± 8% consisted of metabolic products. Reducing glomerular filtration rate by increasing ureteral pressure did not change the percentage of label recovered as angiotensin II or metabolic products (54 ± 8% and 46 ± 7%, respectively). When renal blood flow was reduced, the percentage of label recovered as angiotensin II fell from 47 ± 6 to 29 ± 7 (P < 0.05) and that as metabolites rose from 46 ± 7 to 61 ± 7 (P < 0.05). The heptapeptide des-1-Asp-angiotensin II (angiotensin III) was not found among the metabolites. The results indicate that reductions in glomerular filtration are not important in intrarenal metabolism of angiotensin II in barbiturate-anesthetized dogs. However, changes in renal blood flow markedly affect angiotensin II metabolism. These observations are compatible with the notion that the intrarenal metabolism of angiotensin II in the dog occurs mainly in the vascular compartment, rather than in the proximal tubule, and can be regulated by alterations in renal blood flow. Failure to demonstrate angiotensin III in the renal venous effluent suggests that this peptide is not a major circulating product of intrarenal metabolism of angiotensin II in the dog.

ANGIOTENSIN II is rapidly metabolized by multiple enzyme systems collectively called angiotensinases. These enzymes are present in plasma and in several organs including the kidney.¹⁻³ The rate of disappearance of angiotensin II on passage through the kidney is too rapid to be explained by the action of plasma angiotensinases alone and is probably a function of the activity of intrarenal enzymes.⁴ Although there are some data to suggest that factors affecting renal hemodynamics may play a role in the intrarenal metabolism of this peptide, the extent to which changes in renal blood flow or glomerular filtration might modify metabolism is unknown.⁵⁻⁷ The present study was undertaken to determine whether changes in renal hemodynamics or glomerular filtration rate affect angiotensin II metabolism.

Methods

ANIMAL PREPARATION AND EXPERIMENTAL PROTOCOLS

Male mongrel dogs weighing 15-20 kg were anesthetized with pentobarbital sodium (30 mg/kg, iv), intubated with a cuffed endotracheal tube, and placed on a Harvard positive-pressure respirator. Both femoral veins were cannulated for the infusion of inulin, saline, and additional anesthetic. A femoral artery was cannulated with pentobarbital-anesthetized dogs. However, changes in renal blood flow markedly affect angiotensin II metabolism. These observations are compatible with the notion that the intrarenal metabolism of angiotensin II in the dog occurs mainly in the vascular compartment, rather than in the proximal tubule, and can be regulated by alterations in renal blood flow. Failure to demonstrate angiotensin III in the renal venous effluent suggests that this peptide is not a major circulating product of intrarenal metabolism of angiotensin II in the dog.

From the Departments of Human Development and Physiology, Michigan State University, East Lansing, Michigan, and the Department of Medicine, University of Chicago Medical School, Chicago, Illinois.

Address for reprints: Dr. Michael D. Bailie, Department of Human Development, B 342 Life Sciences, Michigan State University, East Lansing, Michigan 48824.

Received May 20, 1976; accepted for publication January 28, 1977.

output side of a Sigmamotor pump. The left kidney was exposed via an extraperitoneal flank incision and the renal artery and vein were isolated. Both ureters were cannulated with polyethylene tubing. In some experiments, the aorta was exposed above the renal artery and a snare of umbilical tape was placed around the aorta. The dogs then were given heparin sodium (1,000 U/kg, iv). The renal artery was occluded with a bulldog clamp and the renal vein was rapidly (3 minutes or less) cannulated with Tygon tubing. The bulldog clamp was removed and blood was allowed to flow through the tubing into a reservoir from which it was pumped into the jugular vein of the dog at a rate adjusted to keep a constant level in the reservoir. A 23-gauge curved needle attached to a polyethylene catheter was placed directly into the renal artery for injection of labeled angiotensin II. A Carolina Medical Electronics electromagnetic flowmeter probe was placed on the renal artery. The probe was calibrated at the beginning and end of the experiment by taking direct timed collections of the renal venous effluent into a graduated cylinder. Blood was returned to the reservoir after each collection. An infusion of inulin (3% in isotonic saline) was begun at a rate calculated to give arterial blood concentrations between 25 and 35 mg/100 ml. Isotonic saline was infused at approximately 1 ml/kg per min until urine flow rate was between 1 and 2 ml/min.

After renal blood flow had stabilized, one of two protocols was undertaken. In six dogs, synthetic 1-Asp⁻¹²⁵I-4-Tyr⁻⁵-Ile-angiotensin II (¹²⁵I-AII) (New England Nuclear, lot 135-153, 1816 mCi/mg) (25 pmol) was injected as a bolus into the renal artery under control conditions and after a reduction in glomerular filtration rate by elevation of ureteral pressure. The increase in ureteral pressure was accomplished by attaching the ureteral catheter to a pressure transducer and allowing the pressure to rise spontaneously over 20-30 minutes. The sequence of control and experimental injections was alternated with each experi-
ment. A sample of arterial blood was obtained immediately before and after each injection for inulin determination. In a second group of five dogs, synthetic 1-Asp-14C-5-Ile-angiotensin II (14C-AII) (New England Nuclear, lot 307-207, 5 μCi/0.05 ml) (600 pmol) was injected as a bolus into the renal artery during control conditions and after reduction of renal blood flow accomplished by tightening the aortic snare. The sequence of control and experimental injections was alternated with each experiment.

**ANALYTICAL TECHNIQUES AND DATA HANDLING**

In all experiments, approximately 100 ml of the renal venous effluent were collected in 5-ml samples in ice-cold flasks containing 2.6 mM ethylenediaminetetraacetate (EDTA), 1.6 mM dimercaprol, and 3.4 mM 8-OH-quinoline (final concentration). Plasma samples obtained from the systemic circulation contained no detectable radioactivity, ruling out recirculation of appreciable amounts of peptide. There was no evidence of hemolysis in the renal venous effluent. Blood flow were centrifuged immediately at 4°C in a refrigerated centrifuge, and the plasma fraction was removed and frozen until assayed.

The purity of 125I-AII was assessed by high voltage paper electrophoresis and column chromatography on DEAE-Sephadex A-25 as previously described.4,6 The purity of 14C-AII was assessed by two-dimensional peptide mapping.4 Peptide standards for the 14C- and 125I-labeled peptides were prepared as previously described.4 In addition to the heptapeptide, des-1-Asp-angiotensin II (des-1-Asp-AII), which had been synthesized in our laboratory by the solid phase technique, was labeled with 125I by a modification of the chloramine T method,5 purified, and used as an additional peptide standard.

14C-labeled angiotensin II and metabolites were determined by first passing the plasma samples over columns (2 x 50 mm) of Dowex 5-X2 to concentrate the peptides and then subjecting lyophilized eluates to peptide mapping.4 Peptide recoveries from the columns were 90-95%. To determine 125I-labeled peptides, unextracted plasma was subjected to high voltage paper electrophoresis and column chromatography on DEAE-Sephadex A-25.4

Utilizing these techniques, it was possible to distinguish angiotensin II from most of its probable metabolites including des-1-Asp-AII. Products of simultaneous digestion by multiple enzymes were not accounted for in these experiments.

Because the quantity of angiotensin II injected in the 14C-AII experiments was sufficient to cause constriction of the renal vessels, the higher specific activity 125I-AII was utilized for experiments in which glomerular filtration rate alone was decreased. This avoided any possible changes in renal blood flow due to the vasoconstrictor properties of the angiotensin II. While blood flow did decrease following injection of the 14C-labeled peptide in experiments in which it was used, blood flow always was lower following tightening of the snare than during control injections.

Inulin in arterial and renal venous plasma was measured by the diphenylamine method of Walser et al.6 Blood pressure and renal blood flow were taken directly from an oscillograph (Grass polygraph). Glomerular filtration rate (GFR) was calculated from the renal blood flow (RBF), hematocrit (Hct), and extraction of inulin (Eex): \( GFR = \frac{RBF \times (1 - Hct) \times E_{in}}{} \).

**Results**

Elevation of ureteral pressure produced no consistent changes in renal blood flow or systemic blood pressure but did cause a decrease in glomerular filtration rate (Table 1). This 31% decline in glomerular filtration rate was not associated with any alteration in percent breakdown of 125I-AII or distribution of its metabolites. Patterns of separation of peptide fragments by paper electrophoresis and DEAE-Sephadex from a typical experiment were identical before (control) and after (experimental) elevation of ureteral pressure (Fig. 1). After a single circulation through the kidney, 50% of the labeled material appeared as the chymotryptic (1-4) peptide, 30% as intact angiotensin II, and 20% as 125I-Tyr. No peak was seen between the angiotensin II and chymotryptic (1-4) peptide where the des-1-Asp-AII should be located.

Neither percent breakdown of 125I-AII nor the distribution of its metabolites was altered by ureteral occlusion. Of the total labeled material in the renal venous effluent, 52 ± 8% appeared in the form of angiotensin II prior to ureteral occlusion and 54 ± 6% appeared following ureteral occlusion. The distribution of metabolites was: chymotryptic (1-4) peptide, 28 ± 5% prior to and 25 ± 5% following ureteral occlusion; 125I-Tyr, 19 ± 3% prior to and 18 ± 4% following ureteral occlusion.

In contrast, reducing renal perfusion pressure produced substantial increments in the breakdown of 14C-AII in a single passage through the kidney. Table 2 summarizes the effects of tightening the snare around the aorta on renal perfusion pressure and renal blood flow. A 30% reduction in renal perfusion pressure produced a mean decrease in renal blood flow of 45% of control. The reduction in renal blood flow was associated with a decrement in percent of total labeled material recovered as angiotensin II in renal venous blood from 47 ± 6 to 29 ± 4 (P < 0.05). The percent of total labeled material recovered as the two

**Table 1. Effect of Ureteral Pressure Elevation on Renal Hemodynamics**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>BP (mm Hg)</th>
<th>UP (mm Hg)</th>
<th>RBF (ml/min)</th>
<th>GFR (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
</tr>
<tr>
<td>1</td>
<td>140</td>
<td>150</td>
<td>0</td>
<td>105</td>
</tr>
<tr>
<td>2</td>
<td>115</td>
<td>125</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>130</td>
<td>125</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>112</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>110</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>112</td>
<td>115</td>
<td>0 ± 7</td>
<td>249</td>
</tr>
</tbody>
</table>

BP = systemic blood pressure; UP = ureteral pressure; RBF = renal blood flow; GFR = glomerular filtration rate; C = control; E = experimental

* Different from control (P < 0.05).
metabolites increased in proportion [the chymotryptic (5-8) peptide, from 7 ± 2% to 12 ± 2% (P < 0.05); and 14C-Ile, from 46 ± 7% to 61 ± 7% (P < 0.05)]. As in the experiments in which filtration rate was reduced, no des-1-Asp-AII was detected in the renal venous effluent.

Discussion

Inactivation of angiotensin II by kidney has been demonstrated in several laboratories.1-3,5 Osborne and co-workers12 demonstrated that peptide metabolites were recovered in the renal venous effluent, although quantitation of the metabolites was not undertaken. Oparil and Bailie4 studied the metabolism in vivo of angiotensin II by dog kidney and found that 60-80% of labeled angiotensin II was metabolized in a single pass through the kidney.

TABLE 2 Effect of Reduced Renal Perfusion Pressure on Renal Blood Flow

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>RPP (mm Hg)</th>
<th>RBF (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>E</td>
</tr>
<tr>
<td>1</td>
<td>95</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>115</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>95</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
<td>45</td>
</tr>
<tr>
<td>Mean</td>
<td>93</td>
<td>60*</td>
</tr>
<tr>
<td>± SEM</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

RPP = renal perfusion pressure; RBF = renal blood flow.

This breakdown of the peptide was too rapid to be due to plasma angiotensinas.4

Previous studies gave little direct evidence that changes in renal hemodynamics and/or glomerular filtration rate could alter the renal handling of angiotensin II. Pullman et al.,7 in a micropuncture study, found that angiotensin II microinfused directly into the tubular lumen was metabolized rapidly by proximal but not by distal tubules of rats. The design of these experiments did not permit assessment of angiotensin II metabolism in the vascular compartment, the susceptibility of circulating angiotensin II to filtration, or the effects of changes in glomerular filtration rate on the total renal metabolism of angiotensin II.

Leary and Ledingham,6-13 utilizing the isolated perfused rat kidney to study angiotensin II metabolism, found that sodium loading decreased and sodium deprivation increased the ability of the kidney to remove angiotensin II from the vascular bed. In addition, placement of a silver clip on one renal artery affected the ability of both kidneys to remove angiotensin. When the contralateral kidney was left in place, the clipped kidney destroyed angiotensin at a normal rate, and the contralateral kidney inactivated the peptide at a reduced rate. When the contralateral kidney was removed, the solitary clipped kidney showed a reduced ability to remove angiotensin. These alterations in the rate of angiotensin extraction were independent of the development of hypertension. In these experiments, renal blood flow and glomerular filtration rate were not measured, however, and thus altered rates of angiotensin removal could not be related to hemodynamic events.
In the current study, renal hemodynamics and the pattern of enzymatic breakdown of angiotensin II in the kidney were examined in the same preparation. When ureteral pressure was increased, glomerular filtration rate fell without an associated change in renal blood flow, and the maneuver had no effect on angiotensin II metabolism. If hydrolysis of angiotensin II in the proximal tubule had made a major contribution to the renal handling of the peptide, a decrease in glomerular filtration rate should have decreased the percent of labeled material appearing in the renal venous effluent as metabolites. This did not occur, suggesting that the tubule contributes little to the removal of angiotensin II from the renal circulation of the anesthetized dog. This observation was further supported by the earlier finding that only 10% of angiotensin II injected into the renal artery appeared in the urine. In addition, little if any labeled angiotensin II infused in the renal artery appeared in the renal hilar lymph. We cannot rule out the possibility that ureteral occlusion may have induced alterations in tubuloglomerular pressure relationships or renal metabolism that could mask changes in angiotensin handling secondary to a decreased glomerular filtration rate. Nevertheless, it seems unlikely from these data that decreases in glomerular filtration produced by other mechanisms would change angiotensin metabolism in this preparation.

In contrast, reductions in renal blood flow induced by lowering renal perfusion pressure had major effects on the handling of angiotensin by the kidney. Under the conditions of these experiments, there was a 40% reduction in the amount of intact angiotensin II recovered in the venous effluent with a concomitant increase in metabolites. Although the circulation time through the kidney was not estimated in these experiments, the appearance of counts in the venous effluent was delayed by one or two tubes when renal blood flow was reduced. Since each tube contained a constant volume of blood, the renal transit time for the peptide was prolonged. Because the decrease in renal blood flow permitted longer contact between angiotensin II and the vascular wall and resulted in more extensive metabolism of angiotensin II, these results suggest that the major site of enzymatic hydrolysis of angiotensin II in the kidney is in the vascular compartment rather than in the renal tubule. Glomerular filtration rate was not estimated in those dogs in which blood flow was reduced, but it was certainly diminished. However, as indicated by the experiments with reduced glomerular filtration alone, this would not be expected to affect angiotensin metabolism.

These experiments suggest that changes in the rate of blood flow through the kidney may be a physiological regulator of angiotensin II metabolism. The effects of barbiturate anesthesia on basal renal blood flow and glomerular filtration rate must be considered in interpreting results of the current study, however. It has been shown that the administration of pentobarbital anesthesia to conscious dogs causes a fall in total renal blood flow and particularly in flow to the outer cortex, as measured by the xenon washout technique. This effect was more pronounced in sodium-restricted animals and was partially reversed by agents which interrupt the renin-angiotensin system. Hence it was attributed to increased release of endogenous angiotensin II. In the current study, renal blood flow was sensitive to small changes in renal perfusion pressure, possibly reflecting the effects of high intrinsic renal vascular tone following anesthesia and surgical manipulation. Further, administration of 14C-AII to dogs in which renal perfusion pressure was lowered with an aortic snare would be expected to induce an additional reduction in total renal blood flow and superficial cortical flow. These effects may account partially for differences in renal blood flow between the series of animals treated with aortic constriction and given 14C-AII (Table 2) and those in which ureteral pressure was elevated (Table 1) and given 125I-AII.

Since most of the angiotensinase activity of the kidney is found in the superficial cortex, anesthesia-induced changes in total renal blood flow and renal blood flow distribution could affect angiotensin metabolism. Depressed basal renal blood flow and glomerular filtration rate would then mask the effects of further reductions in glomerular filtration. The increase in angiotensin metabolism that occurred when renal blood flow was reduced by constricting the aorta runs counter to this hypothesis, since a further decrease in superficial cortical blood flow and thereby a decrease in angiotensin metabolism would have been expected.

Despite its limitations, the pentobarbital-anesthetized intact dog appears to be a useful model for studying the effects of hemodynamic changes on the renal handling of angiotensin II in physiological and pathological states. Further study will be needed to determine whether renal angiotensin metabolism is enhanced in response to chronic reductions in renal blood flow. Such a mechanism would favor increased renal catabolism of angiotensin II in renal artery stenosis and some varieties of chronic renal parenchymal disease, both of which may be associated with hypertension. If such intrarenal destruction of angiotensin II blunts feedback inhibition of renin release, a mechanism is possible by which the renal hemodynamic abnormality might enhance renin release and thus perpetuate the hypertension.

Finally, it is important to note that the (2-8) heptapeptide was not recovered in these or previous experiments (Fig. 1). Several investigators have suggested that this metabolite may have an important physiological action. Further, Caravaggi and associates recently reported that the (2-8) heptapeptide accounts for about 15% of the circulating angiotensin of the conscious dog. The present experiments fail to demonstrate the (2-8) peptide as a circulating product of the intrarenal metabolism of angiotensin II. The possibility remains that the heptapeptide may be generated from cleavage of angiotensin II by angiotensinases in other peripheral capillary beds or in circulating blood or by the direct action of converting enzyme on des-Asp-angiotensin I. Further studies in vivo will be needed to establish or refute these mechanisms.

Acknowledgments

We thank Keith Crossland, Tously Thiel, Sara Patten, and Noel Bairey for technical assistance and Nancy Fair for preparation of the manuscript.
Effects of Prostaglandin Inhibition on Intrarenal Hemodynamics in Acutely Saline-Loaded Rats

RAINER DÜSING, BERNWARD MELDER, AND HERBERT J. KRAMER

SUMMARY We studied the effect of inhibition of the prostaglandin (PG)-synthesizing enzyme system in female Sprague-Dawley rats following acute expansion of the extracellular fluid volume (ECV). In 57 conscious rats expansion of the ECV with isotonic saline corresponding to an increase in body weight of 10% was induced. Prior to ECV expansion 31 rats received indomethacin (10 mg/kg of body wt) by stomach tube. In six non-ECV-expanded rats indomethacin had no effect on glomerular filtration rate (GFR) and renal plasma flow (RPF). In ECV-expanded rats pretreated with indomethacin, GFR was unaltered but 125 I-hippuran clearance decreased, and filtration fraction significantly increased. Intrarenal 86Rb distribution was similar in control and ECV-expanded rats. Indomethacin caused a slight increase in relative cortical 86Rb activity in non-ECV-expanded rats, but had no effect on intrarenal 86Rb distribution in ECV-expanded rats. No difference in intracortical glomerular perfusion was noted between control and ECV-expanded rats. In indomethacin-treated ECV-expanded rats an increase in relative inner cortical perfusion was observed. Absolute perfusion remained unaltered. Thus the decrease in total RPF was entirely due to decreased perfusion of outer cortical nephrons. Renal prostaglandins therefore may play a permissive role for physical factors to promote renal sodium excretion in acute ECV expansion via changes in intrarenal hemodynamics.

THE ADAPTATION of renal tubular function with its resulting natriuresis following intravenous infusion of isotonic saline may be independent of changes in glomerular filtration rate (GFR) or aldosterone activity. Therefore other determinants of tubular reabsorption of sodium, such as a natriuretic hormone, have been postulated. In addition, the important role of peritubular physical factors, i.e., oncotic and hydrostatic pressure, has been recognized in acute expansion of the extracellular fluid volume (ECV) with isotonic saline. Previous studies concerning the role of changes in intrarenal and intracortical distribution of blood flow in the renal response to acute saline loading have so far led to conflicting results. If such alterations in renal hemodynamics do play a role they may be mediated by intrarenal hormonal action. Thus, infusions of either prostaglandin (PG) A or E have been shown to result in intrarenal hemodynamic changes accompanied by a marked increase in urinary sodium and water excretion in animals and in man (for review see Anderson et al. and Lee et al.). It therefore

REFERENCES


From the Medizinische Universitäts-Poliklinik, Rheinische Friedrich-Wilhelms-Universität, Bonn, West Germany.
Address for reprints: Prof. Dr. H. J. Kramer, Med. Univ. Poliklinik, Wilhelmstr. 35-37, 53 Bonn 1, West Germany.
Received September 8, 1976; accepted for publication January 28, 1977.
Relation of renal hemodynamics to metabolism of angiotensin II by the canine kidney.
M D Bailie and S Oparil

Circ Res. 1977;41:283-287
doi: 10.1161/01.RES.41.3.283

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1977 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4371

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/41/3/283.citation

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/