Renal Extraction of Angiotensin II

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ABSTRACT

Angiotensin II regulates many aspects of renal function and thereby influences long-term blood pressure. The effects of angiotensin II on the kidney have been exhaustively studied; however, the converse (i.e., effects of the kidney on angiotensin II) has received little attention. Accordingly, the focus of this study was to determine whether renal degradation of angiotensin II is regulated by chronic levels of angiotensin II or long-term levels of blood pressure. Twenty hypertensive rats and 22 normotensive rats were treated for 1 week with either vehicle, angiotensin II (50 ng/kg/min, subcutaneously) or captopril (100 mg/kg/day, orally). Right kidney vascular resistance was measured during infusions of angiotensin II into the left renal artery or vena cava at the level of left renal vein. Dose-response data were curve-fitted, and the extraction of angiotensin II by the left kidney was calculated by comparing the doses of angiotensin II required to elicit equal increases in right renal vascular resistance during intravenous versus left intrarenal artery infusions. Renal extraction of angiotensin II was high (mean, 81%) and demonstrated little animal-to-animal variation (coefficient of variation, 23%; standard deviation, 19%). Renal extraction of angiotensin II was independent of hypertension (P = 0.257) or previous chronic exposure to angiotensin II or captopril (P = 0.270), and there was no interaction between hypertension and chronic exposure to angiotensin II or captopril (P = 0.950). We conclude that renal degradation of angiotensin II is constitutively high, is unaffected by chronic levels of arterial blood pressure, and is independent of long-term changes in levels of angiotensin II.

Angiotensin II (Ang II) profoundly influences many aspects of renal function, including renal blood flow (Mitchell and Navar, 1990), glomerular filtration rate (Blantz and Gabbai, 1987), tubular transport (Navar et al., 1999), and renin release (Wagner and Kurtz, 1998). Moreover, by affecting renal function, angiotensin II importantly contributes to the long-term levels of arterial blood pressure (Hall et al., 1999; Granger and Schnackenberg, 2000).

The ability of Ang II to affect renal function is influenced by three primary determinants: 1) the number and type of Ang II receptors, 2) the efficiency of signal transduction processes elicited by Ang II receptors, and 3) the rate of intrarenal degradation Ang II. The first two determinants are pharmacodynamic and have been thoroughly and exhaustively investigated. The third determinant is pharmacokinetic and has received practically no attention.

The present study had three primary goals. The first goal was to determine whether chronic increases or decreases in the levels of Ang II alter the renal degradation of Ang II. Since renal degradation of Ang II limits the ability of Ang II to affect renal function, it would be anticipated that the efficiency of Ang II degradation by the kidneys would be up-regulated when Ang II levels are chronically elevated and down-regulated when Ang II levels are chronically reduced. The second goal was to determine whether long-term levels of arterial blood pressure influence the renal degradation of Ang II. Since long-term exposure to Ang II increases arterial blood pressure (Melaragno and Fink, 1995; Edgley et al., 2001; Kawada et al., 2002) and chronic blockade of Ang II production lowers arterial blood pressure (Antonaccio et al., 1979; Wood et al., 1990), it would be anticipated that the efficiency of Ang II degradation would be increased by hypertension. The third goal was to determine whether an interaction exists between the effects of chronic changes in the levels of Ang II and chronic changes in arterial blood pressure on the efficiency of renal degradation of Ang II.

The design of the present study was straightforward. Spontaneously hypertensive rats (SHR) or normotensive Wistar-Kyoto (WKY) rats were either untreated or treated chronically with either exogenous Ang II or a converting enzyme inhibitor (captopril) to block the formation of Ang II. The doses of Ang II and captopril and the length of treatments were selected to minimize changes in arterial blood pressure so as not to confound changes in levels of Ang II with changes in arterial blood pressure. Next, animals were anesthetized, and Ang II was infused at increasing concentrations into the right renal artery.

ABBREVIATIONS: Ang II, angiotensin II; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto (rat); PE, polyethylene; MABP, mean arterial blood pressure; HR, heart rate; RBF, renal blood flow; RVR, renal vascular resistance.
left renal artery or the vena cava at the level of the left renal vein while renal vascular resistance in the right kidney was monitored as a bioassay. By curve-fitting the dose-response curves, it was possible to calculate the percentage of extraction of Ang II by the left kidney. Since renal extraction of Ang II is largely a function of the ability of the kidney to degrade Ang II, the renal extraction of Ang II serves as a reliable index for the overall ability of the kidney to metabolize Ang II (Hodge et al., 1967; Biron et al., 1968; Bauer et al., 1999). Our results demonstrate that the kidney efficiently extracts Ang II and that the ability of the kidney to extract Ang II is independent of arterial blood pressure or chronic exposure to high or low concentrations of Ang II. These results establish for the first time that the renal degradation of Ang II is constitutively high and independent of key physiological parameters.

Materials and Methods

Animals. Studies used either male SHR (n = 20) or male WKY rats (n = 22) (Taconic Farms, Germantown, NY) that were 14 to 16 weeks of age. The Institutional Animal Care and Use Committee approved all procedures.

Pretreatments. Beginning 1 week before the acute experiments, osmotic minipumps (model 2001; Alza, Palo Alto, CA) infusing either sterile water for injection or Ang II (50 ng/kg/min) dissolved in sterile water for injection were implanted subcutaneously (random assignment). Some rats (random assignment) not receiving Ang II also received captopril (100 mg/kg/day) in their drinking water. Doses of Ang II were selected based on our previous work (Li and Jackson, 1989; Kost and Jackson, 1993).

Surgical Preparation. Rats were anesthetized (Inactin, 90 mg/kg, i.p.; Sigma-Aldrich, St. Louis, MO) and placed on an isothermal pad. Temperature was monitored with a rectal probe thermometer and kept at 37°C with a heat lamp. The trachea was cannulated [polyethylene (PE)-240] to facilitate respiration. Next, a cannula (PE-50) was inserted into the left carotid artery cannula (PE-50), and this cannula was connected to a digital blood pressure analyzer (MicroMed, Louisville, KY) for continuous measurement of mean arterial blood pressure (MABP) and heart rate (HR). Baseline MABPs and HRs were recorded before further surgical manipulation. A PE-50 cannula was placed in the left femoral vein and advanced to the level of the left renal vein, and an infusion of 0.9% saline was begun at 50 μl/min. A transit-time flow probe (model 1 RB; Transonic Systems Inc., Ithaca, NY) was positioned around the right renal artery and connected to a transit-time flow meter (Transonic Systems Inc.) to monitor right renal blood flow (RBF). The left renal artery was thoroughly cleaned, and nerves to the left kidney were removed. A 32-gauge needle connected to a PE-10 catheter was placed into the left renal artery, and an intrarenal artery infusion of 0.9% saline was initiated at 50 μl/min. All branches of the left renal artery and vein were ligated, and any vascular connections through the renal capsule were destroyed. All animals were allowed a stabilization period of 1 h before the experimental protocol was performed.

Protocol. In half the animals, right RBF, MABP, and HR were recorded during the last minute of 5-min intravenous infusions of Ang II (0, 3, 10, 30, and 100 ng/kg/min) infused in 0.9% saline at 50 μl/min, escalating dose-response protocol) followed by 5-min left intrarenal artery infusions of Ang II (0, 10, 30, 100, and 300 ng/kg/min) infused in 0.9% saline at 50 μl/min, escalating dose-response protocol). In the other half of the animals, the same protocol was used, except that dose-response curves to Ang II were elicited with left intrarenal artery infusions first followed by intravenous infusions. Whether the order was intravenous infusions followed by left intrarenal artery infusions or vice versa was determined randomly.

At the end of the experiment, the right kidney was removed and weighed.

Calculation of Percentage of Extraction of Ang II by Left Kidney. The 32-gauge needle was placed directly into the left renal artery, and all extrarenal branches of the left renal artery were ligated. This ensured that Ang II infused into the left renal artery was delivered first to the left kidney with no shunting of the left intrarenal infusion to extrarenal tissue. Also, all branches of the left renal vein and all vascular connections through the renal capsule were destroyed. This ensured that Ang II leaving the left kidney was delivered only to the vena cava at the level of the left renal vein. The intravenous infusions of Ang II were delivered into the vena cava at the level of the left renal vein, i.e., the same level that Ang II escaping extraction by the left kidney was delivered to the vena cava. Therefore, regardless of whether any given molecules of Ang II reached the vena cava by direct infusion or by escaping extraction by the left kidney, the given molecules of Ang II had in common the same pathway from the vena cava to the right kidney. Consequently, extrarenal metabolism of Ang II would be identical regardless of whether Ang II enters the vena cava by direct infusion into the vena cava or by escaping renal extraction. Therefore, extrarenal metabolism is effectively eliminated from the equation to calculate percentage of renal extraction. Ang II receptor excitability in the right kidney would also be independent of whether the Ang II survived renal extraction or was infused directly into the vena cava. Therefore, Ang II receptor excitability is also effectively eliminated from the equation to calculate the percentage of renal extraction. Consequently, in the present experimental paradigm, equal vascular responses of the right kidney to intravenous versus left intrarenal infusions of Ang II imply that the amount of Ang II per unit time entering the vena cava at the level of the left renal artery is identical for the two infusion sites at the given infusion rates. Therefore, the difference between the left intrarenal infusion rate of Ang II and the intravenous infusion rate of Ang II necessary to elicit identical responses in the right kidney represents the rate of Ang II extraction by the left kidney. Dividing this rate by the rate of infusion of Ang II into the left renal artery gives the percentage of extraction of Ang II by the left kidney.

Based on the above considerations, the percentage of extraction of Ang II by the left kidney was calculated for each animal as follows: For each animal, log dose versus changes in right kidney renal vascular resistance (RVR, i.e., RBF divided by MABP) relationships during intravenous and left intrarenal infusions of Ang II were fitted to the equation: response = maximal response/[1 + 10log(ED50)/dose]. The current fits were performed with GraphPad Prism 3.02 (GraphPad Software Inc., San Diego, CA) as described by Motulsky (1999). Fitted relationships were visually inspected, and a response level that was within both the intravenous and left intrarenal log dose-response relationships was selected. This response level was selected such that the log dose-response relationships were as close to parallel as possible at the selected response level. Using the fitted equation, the intravenous and left intrarenal doses of Ang II that caused similar responses, i.e., changes in right kidney RVR, were calculated. The percentage of extraction of Ang II by the left kidney was calculated by the following equation: % extraction = [(left intrarenal dose – intravenous dose)/left intrarenal dose] × 100.

Statistical Analysis. Data were analyzed by one-factor or two-factor analysis of variance, as appropriate, using the Number Cruncher Statistical System (NCSS, Kaysville, UT). The criterion of significance was P < 0.05. Data in figures and tables represent mean ± S.E.M.

Results

Just after anesthesia, but before laparotomy and instrumentation, MABPs in untreated (control), Ang II-pretreated, and captopril-pretreated WKY rats were 133 ± 5, 132 ± 4, and 119 ± 5 mm Hg, respectively. In SHR, just after anesthesia but before laparotomy and instrumentation, MABPs
in untreated (control), Ang II-pretreated, and captopril-pretreated animals were 224 ± 7, 219 ± 7, and 186 ± 8 mm Hg, respectively. These data indicate that the SHR were hypertensive, that the chronic dose of Ang II (50 ng/kg/min) had little effect on MABP, at least in anesthetized animals, and that captopril only minimally lowered MABP.

Panels A, B, and C in Figs. 1 and 2 illustrate representative dose-response relationships for Ang II versus changes in right kidney RVR for the three subgroups of WKY rats (Fig. 1) and the three subgroups of SHR (Fig. 2). In each figure, panels A, B, and C are for untreated, Ang II-pretreated, and captopril-pretreated rats, respectively. These dose-response curves demonstrate three important features: 1) the data points were well described by the equation to which the data were fit (i.e., the experimental data points lie on or close to the fitted curve); 2) the dose-response relationships were shifted to the right by approximately 10-fold when Ang II was infused into the left renal artery versus the vena cava; and 3) the degree to which the dose-response relationship was right-shifted during intrarenal artery infusions appeared approximately the same in WKY rats versus SHR regardless of whether the animals were pretreated or not with Ang II or captopril.

The percentage of extraction of Ang II by the left kidney was calculated for each group as described under Materials and Methods and is shown in panel D of Figs. 1 (WKY rats) and 2 (SHR). The renal extraction of Ang II was not statistically dependent on rat strain (P = 0.257) or previous treatment with Ang II or captopril (P = 0.270). Moreover, there was no significant interaction between rat strain and treatment (P = 0.950) with regard to percentage of extraction of Ang II by the left kidney. Combining all 42 animals, the mean percentage of extraction of Ang II was 81% with a standard deviation of only 19%. Thus, the renal extraction of Ang II was high with little variability.

MABPs, HRs, and right RBFs in instrumented WKY rats during intravenous and intrarenal artery infusion of Ang II are provided in Tables 1 and 2, respectively. MABPs, HRs, and right RBFs in instrumented SHR during intravenous and intrarenal artery infusion of Ang II are provided in Tables 3 and 4, respectively. MABP was lower in WKY rats and SHR after laparotomy, extensive surgical manipulation, and instrumenta- tion. Importantly, intravenous and intrarenal artery infusions of Ang II, at the doses used in the current study, did not affect HR and caused only moderate increases in MABP and modest decreases in right RBF. Thus, the doses of Ang II used in the current study did not cause marked hemodynamic disturbances.

**Discussion**

The degradation of Ang II by the kidney can be determined by two approaches. One approach is to incubate renal tissue with Ang II and measure the disappearance of Ang II from the incubation medium. This strategy has the limitation that the presentation of Ang II to the isolated renal tissue in vitro...
is quite different compared with the delivery of Ang II by the systemic circulation in vivo. Therefore, in the present study, we chose the alternative approach of measuring the renal extraction of Ang II by the kidneys in vivo, a method that provides an index of renal degradation of Ang II under more physiological conditions. Although Ang II peptides are also filtered so that renal extraction cannot be equated one-to-one with renal degradation (i.e., some Ang II is removed by glomerular filtration), the renal extraction of Ang II is much greater than the filtration fraction, a finding consistent with the conclusion that the major determinant of renal extraction of Ang II is renal degradation of Ang II. This conclusion is confirmed by the marked effects of amastatin, an inhibitor of aminopeptidases involved in Ang II degradation, on the renal effects of infused Ang II (Bauer et al., 1999).

Renal extraction of Ang II can be determined by two approaches. In this regard, one approach is to take arterial and renal venous blood samples and to measure analytically the

### TABLE 1

<table>
<thead>
<tr>
<th>Intravenous Infusion Rate of Angiotensin II (ng/kg/min)</th>
<th>0</th>
<th>10</th>
<th>30</th>
<th>100</th>
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<tbody>
<tr>
<td>WKY, control (n = 7)</td>
<td>MABP: 97 ± 5</td>
<td>102 ± 4</td>
<td>104 ± 7</td>
<td>116 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>HR: 377 ± 11</td>
<td>379 ± 12</td>
<td>384 ± 13</td>
<td>381 ± 12</td>
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<tr>
<td></td>
<td>Right RBF: 8.1 ± 1.8</td>
<td>8.3 ± 1.9</td>
<td>8.1 ± 1.9</td>
<td>6.9 ± 1.7</td>
</tr>
<tr>
<td>WKY, pretreated with angiotensin II (n = 7)</td>
<td>MABP: 97 ± 2</td>
<td>99 ± 3</td>
<td>100 ± 4</td>
<td>115 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HR: 397 ± 11</td>
<td>395 ± 12</td>
<td>395 ± 12</td>
<td>395 ± 12</td>
</tr>
<tr>
<td></td>
<td>Right RBF: 6.9 ± 1.5</td>
<td>6.7 ± 1.4</td>
<td>6.3 ± 1.3</td>
<td>5.1 ± 1.1</td>
</tr>
<tr>
<td>WKY, pretreated with captopril (n = 8)</td>
<td>MABP: 84 ± 3</td>
<td>86 ± 4</td>
<td>89 ± 5</td>
<td>100 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HR: 369 ± 15</td>
<td>368 ± 14</td>
<td>368 ± 13</td>
<td>369 ± 12</td>
</tr>
<tr>
<td></td>
<td>Right RBF: 4.6 ± 0.8</td>
<td>4.7 ± 0.8</td>
<td>4.4 ± 0.8</td>
<td>3.9 ± 0.6</td>
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</table>

<sup>a</sup> P < 0.05 compared with 0 (baseline).

**Fig. 2.** Panels A, B, and C, relationship in SHR between log dose of Ang II and change in renal vascular resistance (ΔRVR) of the right kidney during intravenous infusions (●) versus infusions into the left renal artery (△). Panels A, B, and C represent a single experiment for untreated (control), chronically Ang II-pretreated, and chronically captopril-pretreated SHR. Panel D, percentage of extraction of Ang II across the left kidney in SHR in untreated (control, n = 7), chronically Ang II-pretreated (Ang II, n = 6), and chronically captopril-treated (captopril, n = 7) animals. Values represent means ± S.E.M.
concentrations of Ang II across the renal circulation. However, this approach is not practical in small animals because of the large blood sample volumes necessary to measure plasma levels of Ang II by currently available methods. Therefore, in the current study we chose the alternative approach of bioassay. As reported recently by us (Jackson and Herzer, 2001), the renal circulation is the most sensitive of all vascular beds to the acute vasoconstrictive effects of Ang II. Therefore, we used changes in right kidney RVR as a bioassay for the concentration of Ang II in the blood supplying the right kidney. The underlying assumption of this technique is that equal changes in right kidney RVR indicate equal changes in Ang II concentrations entering the right renal artery. This assumption seems reasonable, with one caveat; it is conceivable that intrarenal artery infusions of Ang II into the left kidney could release vasoactive factors that survive clearance by the lungs at sufficient concentrations to affect right kidney RVR. However, the percentage of extraction of Ang II by the kidney determined in the present study (81%) is very similar to the percentage of extraction of Ang II by the kidney measured in larger animals using analytical methods, e.g., 70% in dogs (Reams et al., 1990). Therefore, it appears that the underlying assumption of our bioassay approach is valid.

The results of the present study strongly support the conclusion that the renal degradation of Ang II is not different in
normotensive versus hypertensive rats. Moreover, our results indicate that chronically increasing Ang II levels by administering exogenous Ang II or chronically decreasing Ang II levels by administering an angiotensin-converting enzyme inhibitor does not modify in any significant way the renal degradation of Ang II. Finally, our results are consistent with the conclusion that normotensive and hypertensive rats are similar with respect to renal degradation of Ang II regardless of whether chronic levels of Ang II are high, normal, or low.

In previous studies, we (Li and Jackson, 1989; Kost and Jackson, 1993; Kost et al., 1994) and others (Arendshorst et al., 1990; Chatziantoniou et al., 1990) have reported that the renal vasculature in SHR is more sensitive to Ang II compared with WKY rats and that this enhanced renal sensitivity to Ang II contributes to the pathophysiology of hypertension in this model of genetic hypertension. The enhanced renal sensitivity to Ang II in SHR is in part due to defects in the $G_a$ (Chatziantoniou et al., 1995) and $G_i$ (Jackson, 1994; Gao et al., 2003) signal transduction pathways but apparently is independent of changes in the number of angiotensin receptors (Gao et al., 2003). The findings of the present study strongly suggest that enhanced renal sensitivity to Ang II in SHR is also independent of renal degradation of Ang II. Thus, it appears that the enhanced renal sensitivity to Ang II in SHR is mediated by changes in signal transduction.

In summary, the present study defines the renal extraction of Ang II in normotensive and hypertensive rats under control conditions and following chronic treatment with a subpressor dose of Ang II or with a mildly antihypertensive dose of captopril. Our results indicate that the renal extraction of Ang II, and by inference the renal degradation of Ang II, is not different in normotensive versus hypertensive rats either under control conditions or when Ang II levels are chronically manipulated. Finally, our results support the conclusion that the renal degradation of Ang II is constitutively high. The constitutively high rate of Ang II degradation by the kidney may serve to protect this organ from the powerful influences of Ang II on renal hemodynamics and excretory function.

References


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