Intrarenal Conversion of Angiotensin I to Angiotensin II in the Dog

By Joseph Di Salvo, Aron Peterson, Cheryl Montefusco, and Margaret Menta

ABSTRACT

Effects of close intra-arterial injections of angiotensins I and II on changes in renal blood flow and renal vascular resistance were studied in dogs anesthetized with pentobarbital sodium. In the first series of experiments, angiotensin I (0.375 µg) or angiotensin II (0.13 µg) decreased renal blood flow and pressures in a small renal cortical vein and the renal vein. Although renal vascular resistance was markedly increased by angiotensins I or II, these increases did not appear to involve renal venous segments. Responses to angiotensin I occurred when the injected agonist was not allowed to reach the systemic circulation, and were virtually abolished during infusion of SQ 20475, a synthetic pentapeptide (Pyr-Lys-Try-Ala-Pro) that inhibits enzymatic conversion of angiotensin I to angiotensin II. In the second series of experiments, renal vascular responses to intra-arterial injections of angiotensin I (0.375-3.75 µg) and angiotensin II (0.13-1.00 µg) were studied. Responses to angiotensin I, but not to angiotensin II, were significantly attenuated by SQ 20475. The results suggest that similar renal vascular segments respond to angiotensins I and II. Responses to angiotensin I are probably due to its intrarenal conversion to angiotensin II. Such conversion appears to occur to the extent of about 4%.

KEY WORDS renal blood flow angiotensin-converting enzyme renal venous resistance renal vascular resistance vasoconstriction segmental resistance

Recently, Franklin et al. (1) reported that the decapeptide angiotensin I produces renal vasoconstriction in dogs. They suggested that the response was due to intrarenal conversion of angiotensin I to the markedly vasoactive octapeptide angiotensin II. The conversion reportedly occurred to the extent of about 20%. These findings contrast with those reported by Ng and Vane (2, 3), which suggest that intrarenal conversion of angiotensin I to angiotensin II is minimal.

Clearly, the renal vascular response to angiotensins I and II, with particular reference to intrarenal conversion of angiotensin I to angiotensin II, merits further investigation. Such information is of interest because of the suggested intrarenal role of the renin-angiotensin system in regulating renal function (4, 5).

In the present study, renal vascular responses to intra-arterial injections of angiotensins I and II were examined in dogs in the presence and absence of an inhibitor of angiotensin-converting enzyme. The results show that (1) angiotensins I and II produce marked increases in renal vascular resistance that do not appear to involve renal venous segments, and (2) the response to angiotensin I is due to an intrarenal mechanism. The findings suggest that intrarenal conversion of angiotensin I to angiotensin II occurs to the extent of about 4%.

Methods

Experiments were performed on 21 mongrel dogs of either sex (7-9 kg) anesthetized with pentobarbital sodium (30 mg/kg, iv). The
animals were intubated with a cuffed endotracheal tube and allowed to breathe spontaneously. Arterial blood pressure was measured with a Statham P23Db pressure transducer on a catheter inserted into the left femoral artery and advanced into the abdominal aorta until its tip was close to the renal artery.

SERIES I (TWELVE DOGS)

Blood leaving the kidney was shunted through an extracorporeal circuit and returned to the animal by methods similar to those described by Gazitua et al. (6, 7). The left renal vein was cannulated with a PE320 catheter, which was connected to a short segment of rubber tubing joined to a Y-tube. One end of the Y was connected to a cannula inserted into the left jugular vein. The other end of the Y, which could be opened to the atmosphere, was clamped shut. By simultaneously releasing the clamp and occluding the end of the Y leading to the jugular vein, blood draining the kidney could be diverted to a graduated receptacle. Blood collected in this way was returned to the animal through a gravity-fed reservoir in the left femoral vein.

A PE10 catheter was inserted into the extracorporeal circuit until its tip was in a small cortical vein, and small vein pressure was measured with a Statham P23De pressure transducer. Blood was freely aspirated from this catheter, and flushing with saline caused an abrupt rise in pressure that quickly returned to its initial value, suggesting that a true lateral pressure was measured (7, 8). Renal vein pressure was measured with a Statham P23De pressure transducer on a second catheter inserted into the extracorporeal circuit and advanced until its tip was in the renal vein. The positions of the renal and small vein catheters were confirmed at necropsy.

A noncannulating electromagnetic flow sensor of the gated sine-wave type (Statham M4001) was placed on the renal artery to measure rapid changes in renal blood flow (RBF). Changes in small vein and renal vein pressures and RBF in response to intra-arterial injections (0.1 ml) of angiotensin I (0.37 μg, Schwarz BioResearch Inc. [Asp¹, Ileu⁸-]), 0.65 μmole peptide/mg) or angiotensin II (0.13 μg, Ciba [Asp¹, Val⁵-], 0.78 μmole peptide/mg) were studied. Three resistances were calculated, according to the following formulas:

\[
\text{Total Resistance} (\frac{R_t}{\text{RBF}}) = \frac{(P_a - P_v)}{\text{RBF}}
\]

\[
\text{Resistance 1} (\frac{R_1}{\text{RBF}}) = \frac{(P_a - P_{sv})}{\text{RBF}}
\]

\[
\text{Resistance 2} (\frac{R_2}{\text{RBF}}) = \frac{(P_{sv} - P_v)}{\text{RBF}},
\]

where \(P_a\) is arterial blood pressure, \(P_v\) is renal blood pressure, and \(P_{sv}\) is small vein pressure.

Calculation of the segmental resistances, \(R_1\) and \(R_2\), rests on the assumptions that (1) the measured small vein pressure accurately reflects small vein pressure in all renal veins of similar size, (2) sufficient vein-to-vein anastomoses exist upstream to the catheter tip so that a true lateral pressure is measured, and (3) changes in RBF are distributed equally throughout the kidney. Clearly, such assumptions are not always valid, particularly since the renal circulation is a portal one, and redistribution of intrarenal blood flow is known to occur (9, 10). Nevertheless, calculations of \(R_1\) and \(R_2\) allow some insight into localization of resistance changes to the arterial (\(R_1\)) versus the venous (\(R_2\)) segments of the renal circulation (6, 7, 11, 12).

In six animals of the series, responses to angiotensins I and II were studied before and during intra-arterial infusion of SQ 20475 (Pyr-Lys-Try-Ala-Pro, 50 μg/kg min⁻¹), a synthetic pentapeptide originally isolated from Bothrops jararaca venom (BPP5a) that inhibits enzymatic conversion of angiotensin I to angiotensin II (13–15). The pentapeptide was dissolved in saline, adjusted to pH 7.4, and infused for 20 minutes prior to angiotensin injections. The infusion was continued until all injections had been completed.

In the six remaining animals of the series, renal responses to angiotensins I and II were studied before and during intra-arterial infusion of SQ 20694 (Pyr-Lys-D-Try-Ala-Pro, 50 μg/kg min⁻¹) a structural analog of SQ 20475, which, at the dose given, does not appear to inhibit conversion of angiotensin I (unpublished observations). In these animals, responses to angiotensin I were also studied while the renal venous effluent was collected, so that none of the injected agonist reached the systemic circulation. To prevent changes in blood volume and arterial pressure during the blood collection, 6% dextran was infused into the jugular vein at a rate about equal to the renal venous outflow. Collection of the renal venous effluent was maintained for 1.5 minutes to provide sufficient time for demonstrating renal vascular action of angiotensin I, and to minimize changes in hematocrit which could occur during volume replacement with dextran (16).

Student's paired t-test was used to evaluate angiotensin responses before and after pentapeptide blockade (17). All directly measured parameters were continuously monitored on an Offner direct-writing oscillograph.

SERIES II (NINE DOGS)

The methods used for measuring changes in RBF have been described previously (18, 19). Briefly, the left kidney was exposed via a flank incision. A noncannulating electromagnetic flow
sensor was placed around the renal artery, care being taken not to damage the renal nerves. The zero-flow base line was established at the beginning of each experiment and checked at its termination by momentarily occluding the artery distal to the flow sensor. The flow sensor was calibrated at the end of each experiment by cannulating the renal vein and diverting renal venous outflow to a graduated cylinder for 30 seconds.

Angiotensin I was dissolved in saline, adjusted to pH 7.2–7.4, and injected rapidly (0.05–0.5 ml) into the renal artery through a 25-gauge needle inserted proximal to the flow sensor. The doses of angiotensin I tested were 0.37, 0.75, 1.50, 2.25, 3.0, and 3.75 μg. The maximal change in RBF occurring 5–15 seconds after injection and in the absence of significant changes in arterial pressure was taken as the principal response. Changes in RBF were expressed as percent of control flow measured just prior to injection of the agonist (18, 19). Responses to angiotensin II were studied in identical fashion at doses of 0.13, 0.25, 0.75, and 1.0 μg. Control saline injections were made in all experiments. Dose-response curves were prepared, using the least-squares method (20). The ratio of equipotent doses of angiotensin I to angiotensin II, with respect to producing a decrease in RBF, multiplied by 1.25 was used to calculate percent conversion of angiotensin I to angiotensin II (21, 22). The factor 1.25 corrects for the difference in molecular weight between angiotensins I and II.

In six animals of the series, responses to angiotensin I were studied before and during infusion of the converting-enzyme antagonist, SQ 20475, as described for series I. Data obtained were plotted in double-reciprocal fashion, according to the Lineweaver-Burk method (23). Specifically, the reciprocal of the decrease in RBF was plotted on the ordinate, against the reciprocal of agonist dose (μg) on the abscissa. As described by Chen and Russel (24), the curve so generated conforms to the following equation: 1/V = 1/Vmax + (Ks/Vmax) (1/S), where V is response (% decrease in RBF), 1/Vmax (the ordinate intercept) is the reciprocal of the maximal decrease in RBF, Ks is the dissociation constant of the agonist-receptor complex, or enzyme-substrate complex (23, 25), and S is dose of agonist (μg). In the present study, Ks was calculated from the slope of the curve (25). Data plotted in this way provide insight into the mechanism of action of the antagonist (24–26). We recognize that applicability of Lineweaver-Burk double-reciprocal analysis to systems in vivo is limited, since accurate estimation of Ks depends on the concentration of angiotensin I at the site of the converting enzyme, as well as on the amount and rate of product (angiotensin II) formed. In the present study, such information is not available since (1) the concentration of angiotensin I at the converting enzyme site is probably not the same as the dose injected, (2) rapid elimination of the injected agonist and degradation of the presumed product (angiotensin II) are not determinable, and (3) the decrease in RBF during injection of angiotensin I may not be uniform throughout the renal mass (9, 10).

All measured parameters are given as the mean ±1 SE. In all experiments, the injection schedule was randomized with respect to agonist and dose level. Sufficient time (5–10 minutes) was allowed between injections so that tachyphylaxis to angiotensins I or II did not develop. The contralateral kidney was left intact and untouched in both series I and II experiments.

Results

SERIES I

Control RBF for animals in this series was 67 ± 3 ml/min at the beginning of experiments and 60 ± 4 ml/min when the experiments were terminated. Corresponding values for systemic arterial blood pressure were 138 ± 5 and 133 ± 5 mm Hg, respectively.

Intra-arterial injection of angiotensin II (0.13 μg) or angiotensin I (0.37 μg) caused marked decreases in renal vein and small-vein pressures and renal blood flow (Fig. 1). These responses always occurred in the absence of significant changes in arterial blood pressure.

\[1/V = 1/V_{\text{max}} + (K_s/V_{\text{max}}) (1/S)\]

**FIGURE 1**

Effects of angiotensin I and angiotensin II. Arrows indicate time of injection of agonist. (See text.)

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Renal vascular responses in six dogs to angiotensin II in the presence and absence of SQ 20475 (0.75 μg/ml RBF kg⁻¹). Small vertical bars represent 1 se. Control values are given above the letter C on the abscissa, and the experimental value is given above the symbol An. Responses to angiotensin II were not altered by SQ 20475.

Angiotensin I (Fig. 3) caused changes in renal vein and small vein pressures, RBF, Rti, and Rti that were directionally similar, but of smaller magnitude, to those caused by angiotensin II. Angiotensin I, like angiotensin II, caused no significant change in Rvi. Thus, angiotensin I increased Rti from 2.05 ± 0.1 at control to 2.94 ± 0.2 mm Hg/ml min⁻¹ (P < 0.001) in the absence of SQ 20475. In contrast, calculated Rti was only 2.14 ± 0.1 mm Hg/ml min⁻¹ in response to the same dose of angiotensin I during infusion of SQ 20475.

Collection of renal venous blood during and 1.5 minutes after intra-arterial injection of angiotensin I, so that none of the injected agonist reached the systemic circulation, did not alter the systemic arterial pressure or heart rate. The decrease in RBF was rapid in onset (2-5 seconds), reached a maximum in 5-15 seconds, and returned to control flow level in 1-3 minutes. In all animals, decreases in renal vein and small vein pressures and RBF in response to either angiotensin I or angiotensin II were coincident in time. Although, in this series of experiments, the administered dose of angiotensin II was always less than that of angiotensin I, the magnitudes of responses to angiotensin II were always greater than those to angiotensin I.

Angiotensin II (Fig. 2) decreased small vein pressure from the control value of 40 ± 2 to 18 ± 2 mm Hg (P < 0.001). Renal vein pressure and RBF were significantly decreased, and systemic arterial blood pressure was not changed. Resistance to blood flow (Rf) increased from its initial value of 2.05 ± 0.1 to 4.50 ± 0.5 mm Hg/ml min⁻¹ (P < 0.001). Rvi increased significantly (P < 0.001), whereas Rvi was unchanged. Renal vascular responses to angiotensin II were, not significantly altered when studied during intra-arterial infusion of SQ 20475 (50 μg/kg min⁻¹). If average RBF is taken as 67 ml/min, this infusion rate of SQ 20475 corresponds to 0.75 μg SQ 20475/ml RBF kg⁻¹.

Angiotensin I (Fig. 3) caused changes in renal vein and small vein pressures, RBF, Rti, and Rti that were directionally similar, but of smaller magnitude, to those caused by angiotensin II. Angiotensin I, like angiotensin II, caused no significant change in Rvi. Thus, angiotensin I increased Rti from 2.05 ± 0.1 at control to 2.94 ± 0.2 mm Hg/ml min⁻¹ (P < 0.001) in the absence of SQ 20475. In contrast, calculated Rti was only 2.14 ± 0.1 mm Hg/ml min⁻¹ in response to the same dose of angiotensin I during infusion of SQ 20475.

Collection of renal venous blood during and 1.5 minutes after intra-arterial injection of angiotensin I, so that none of the injected agonist reached the systemic circulation, did not affect the systemic arterial pressure or heart rate.
not alter the response to angiotensin I (Fig. 4, right). Injection of angiotensin I increased Rt from its control of 2.10 ± 0.1 to 3.05 ± 0.1 mm Hg/ml min⁻¹ (P < 0.001), even though none of the injected agonist entered the systemic circulation. When the injection was repeated, while renal venous blood returned to the animal, the same dose of angiotensin I increased Rt to 3.15 ± 0.15 mm Hg/ml min⁻¹.

During infusion of the pentapeptide analog of SQ 20475, SQ 20694 (50 µg/kg min⁻¹), renal vascular responses to either angiotensin I or angiotensin II were not changed. Infusion of either SQ 20475 or SQ 20694 did not alter arterial, renal vein or small vein pressures or RBF.

SERIES II

Systemic arterial blood pressure for animals in this series averaged 140 ± 5 mm Hg at the start of experiments and was 134 ± 7 mm Hg at termination. Corresponding values for renal blood flow were 86 ± 4 and 77 ± 6 ml/min.

In all animals, as in series I, decreases in RBF in response to intra-arterial injections of either angiotensin I (0.375–3.75 µg) or angiotensin II (0.13–1.00 µg) were rapid in onset and short in duration, and occurred in the absence of significant changes in systemic arterial pressure. Blood-flow responses to either angiotensin I or angiotensin II were directly proportional to log dose throughout the range studied (Fig. 5). Although the dose-response curves for angiotensins I and II were parallel, angiotensin II was consistently more potent than angiotensin I in producing renal vasoconstriction. Angiotensin II at a dose of 0.13 µg decreased RBF to 40 ± 6% of control, whereas 3.75 µg of angiotensin I was required to produce the same change in flow. Clearly, angiotensin II is about 30 times as potent as angiotensin I in reducing RBF. If the response to angiotensin I is due to intrarenal enzymatic conversion to angiotensin II, our results correspond to about 4.3% conversion. That is, the dose of angiotensin II (0.13 µg) divided by the dose of angiotensin I (3.75 µg) that produced the same decrease in RBF (40% of control), when multiplied by the factor 1.25 (correcting for the different molecular weights of angiotensins I and II) yields a value of 4.3% conversion.
TABLE 1

<table>
<thead>
<tr>
<th>Angiotensin I (µg)</th>
<th>% of Control RBF Before SQ 20475</th>
<th>During SQ 20475</th>
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<tbody>
<tr>
<td>0.375</td>
<td>71 ± 3</td>
<td>92 ± 3*</td>
</tr>
<tr>
<td>0.750</td>
<td>57 ± 3</td>
<td>68 ± 4*</td>
</tr>
<tr>
<td>2.250</td>
<td>42 ± 4</td>
<td>46 ± 4</td>
</tr>
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Values are means ± 1 SE for seven dogs.

*P < 0.01, relative to RBF response before SQ 20475.

SQ 20475 (50 µg/kg min⁻¹) significantly attenuated the renal-vasoconstrictor response to 0.375 or 0.75 µg of angiotensin I (Table 1). If average RBF for this series of experiments is taken as 85 ml/min, the dose corresponds to about 0.56 µg SQ 20475/ml RBF kg⁻¹. Double-reciprocal Lineweaver-Burk plots show that the ordinate intercepts (1/Vmax) generated from angiotensin I data in the presence (0.014%) and absence (0.011%) of SQ 20475 were similar (Fig. 6). In contrast, calculated Ks values were 0.65 µg in the presence, and 1.46 µg in the absence, of SQ 20475. The Ks value calculated for angiotensin II was 0.09 µg.

Discussion

Levels of RBF observed in the second series of experiments are in accord with previously reported values (19, 27). RBF in series I was lower. This difference could be due simply to the more extensive surgical procedures in series I. However, in either series, RBF and arterial blood pressure levels were not significantly altered throughout the experimental period (2-3 hours). Further, tachyphylaxis to angiotensins I or II did not develop. These findings suggest that all animals were adequately responsive to intra-arterially injected agonists during each experiment.

The results of the present study show that (1) angiotensins I and II produce marked increases in renal vascular resistance that do not appear to involve renal venous segments; (2) responses to angiotensin I are demonstrable even when the injected agonist is not permitted to reach the systemic circulation, where it may be converted to angiotensin II; (3) responses to angiotensin I but not those to angiotensin II are inhibited by SQ 20475, which inhibits angiotensin-converting enzyme, and (4) angiotensin II is about 30 times as potent as angiotensin I in producing renal vasoconstriction. These findings suggest that the renal vascular response to angiotensin I is due to its intrarenal enzymatic conversion to angiotensin II, and that such conversion occurs to the extent of about 4%. Further, the data are consistent with the hypothesis that inhibition of conversion of angiotensin I to angiotensin II by SQ 20475 probably involves a mechanism of enzymatic competitive inhibition.

Zimmerman et al. (11) reported that the rise in canine renal vascular resistance (Rt) produced by angiotensin II occurs predominantly in the arterial side (R1) of the renal circulation, whereas changes in renal venous resistance (R2) are minimal. Our findings with angiotensin II (Figs. 2 and 4) are consistent with those reported by Zimmerman et al. The present study shows that the rise in resistance produced by angiotensin I also occurs predominantly in the arterial segments of the renal circulation (Figs. 3 and 4). Indeed, more than 90% of the increase in Rt produced by either angiotensin I or II was
attributable to the rise in $R_1$, which suggests that both agonists act on the same or similar vascular segments. Decreases in renal vein and small vein pressures that occurred in response to angiotensins I and II seem to be due to the decrease in RBF.

The demonstration that renal vasoconstrictor responses to angiotensin I were rapid in onset, and that they occurred in the absence of significant changes in arterial pressure, supports the hypothesis that the response is due to an intrarenal mechanism (Fig. 1). Further support for this view is derived from the finding that angiotensin I still produced renal vasoconstriction when it was excluded from the systemic circulation (Fig. 4). Therefore, it seems that angiotensin I constricts the renal vasculature by acting directly on renal vascular smooth muscle, or as a result of intrarenal conversion to angiotensin II, or both.

The pentapeptide SQ 20475 markedly inhibited vasoconstriction caused by angiotensin I (Fig. 3) but not that caused by angiotensin II (Fig. 2). Therefore, inhibition of responses to angiotensin I with SQ 20475 at the dose used does not appear to be due to a nonspecific pharmacologic effect which could alter smooth muscle responsiveness. SQ 20475 inhibits angiotensin-converting enzyme (13-15). Our results are thus consonant with the view that renal vasoconstriction produced by angiotensin I is largely due to its intrarenal enzymatic conversion to angiotensin II. Vasoconstrictor responses to angiotensin I in the presence of SQ 20475 could result from incomplete inhibition of the converting enzyme, from a small degree of inherent angiotensin I vasomotor activity, or from both.

Double-reciprocal plots of RBF decreases obtained with graded doses of angiotensin I in the presence and absence of SQ 20475 facilitate examination of the mechanism of inhibition by SQ 20475 (24-26). Presumably, $K_s$ values derived from such data are directly related to the dissociation constant of the converting enzyme-angiotensin I complex (25, 26). Calculated $K_s$ values in the presence (1.46 μg) and absence (0.65 μg) of SQ 20475 were widely different, whereas the ordinate intercepts ($1/V_{max}$) were very similar (Fig. 6). These findings are suggestive of a predominantly competitive type of enzyme inhibition (24-26).

Further, SQ 20694, which differs from SQ 20475 only in that the third amino acid residue (Try) is replaced with its D-isomer, did not alter the effects of either angiotensin I or II (Fig. 4). This shows that inhibition of the effects of angiotensin I by SQ 20475 is highly specific, a finding that is in accord with the concept of competitive inhibition (25, 26).

The marked sensitivity of the renal vasculature to angiotensin II is consistent with previous studies (17, 28). Since angiotensin II is about 30 times more potent than angiotensin I (Fig. 5), maximal intrarenal conversion of angiotensin I to angiotensin II could occur to the extent of 4%. However, the true extent of conversion is probably lower than the estimated 4%, since angiotensin I reportedly possesses a minute degree of inherent vasoactivity (15). Our conversion level agrees with estimates from Vane's laboratory (2, 3, 15), but disagrees with the high conversion level (18%) reported by Franklin et al. (1). The method employed by the Franklin group for estimation of angiotensin conversion, however, involved comparison of RBF decreases in response to different, but fixed, doses of angiotensins I and II. In contrast, bioassay procedures require that potency comparisons between agonists (angiotensins I and II), or estimates of percent conversion, be evaluated at doses that elicit responses of equal magnitude (21, 22, 25, 26), because, mathematically, agonist dose-response relations follow a sigmoid relation (22, 25, 26). In the present study, we calculated percent conversion at doses of angiotensins I and II that produced equivalent decreases in RBF.

Our results also differ from those reported by Oparil et al. (29) indicating that renal conversion of angiotensin I to angiotensin II occurs on the order of 7-10%. However, they used a radioimmunoassay technique for estimating angiotensin I conversion, whereas we used direct measures of renal blood flow changes produced by equipotent doses of
RENAL CONVERSION OF ANGIOTENSIN I


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