In Vivo Comparison of Renal and Femoral Vascular Sensitivity and Local Angiotensin Generation

Tongchuan Li and Ben G. Zimmerman

Experiments were conducted to compare the relative importance of the local renin-angiotensin systems in the rabbit renal and femoral vascular beds and their functional role in hemodynamic regulation. Angiotensin I (Ang I) (0.15 μg/kg i.v.) elevated mean arterial blood pressure by 18 ± 1 mm Hg in the renal experimental group and 19 ± 1 mm Hg in the femoral experimental group; it decreased renal blood flow by 35 ± 3% but increased femoral blood flow by 31 ± 8%. All these effects were blocked by intravenous administration of captopril (2 mg/kg bolus injection plus 1 mg/kg/hr). Captopril also lowered mean arterial pressure by 17 ± 3 and 16 ± 2 mm Hg in the renal and femoral experimental groups, respectively, and it increased renal blood flow by 32 ± 10% but reduced femoral blood flow by 21 ± 4%. As a result, renal vascular resistance was decreased by 36 ± 5%, but femoral vascular resistance remained unchanged. After captopril, plasma angiotensin II (Ang II) levels were decreased and Ang I levels increased in the two groups. The renal venous-arterial difference of Ang I was increased by captopril, but the femoral venous-arterial difference of Ang I was not, suggesting greater generation of Ang I in the kidney. In a separate group of bilateral nephrectomized rabbits, plasma Ang II levels as well as mean arterial pressure, femoral blood flow, and femoral vascular resistance were not changed by intravenous administration of captopril. These results indicate that in the rabbit femoral vascular resistance is not controlled by the renin-angiotensin system directly, and the local renin-angiotensin system in the kidney is more important in regulating renal vascular tone. (Hypertension 1990;15:204-209)

The concept of a tissue renin-angiotensin system has gradually evolved over the past 10 years. Many studies have provided evidence for the local generation of angiotensin within different tissues, including brain, kidney, adrenal gland, testis, and arterial wall, through mechanisms that appear to operate independently of the circulating renin-angiotensin system. Angiotensin I (Ang I) or angiotensin II (Ang II) are generated in and released from isolated rat mesenteric arteries, rat kidney, rat hind legs, but there is little evidence for a relation between this locally generated Ang II and the regulation of systemic blood pressure or local blood flow. In the present study, experiments were conducted in vivo to compare the local renin-angiotensin systems in the lapine renal and femoral vascular beds with respect to their relative activity and possible role in hemodynamic regulation. The rabbit was the species chosen for study because of its highly responsive renin-angiotensin system. A high dose of captopril was used intravenously to inhibit angiotensin converting enzyme. The effects of captopril were determined on plasma immunoreactive Ang I and Ang II levels, renal blood flow (RBF), femoral blood flow (FBF), and the response to intravenously administered Ang I. The venous concentration of immunoreactive Ang I and Ang II of the two vascular beds was used as a means of assessing local production of angiotensins. The femoral vascular bed was studied in a separate group of nephrectomized rabbits to see if the converting enzyme inhibitor captopril exerted any effect on the arterial and femoral venous plasma Ang II level and hemodynamic parameters after removal of the systemic renin-angiotensin system.

Methods

Acute Experiment

Twenty-four male New Zealand white rabbits weighing 3.0–4.2 kg were divided into three groups, 11 in group 1 (renal experiment), seven in group 2 (femoral experiment), and six in group 3 (nephrec-
Anesthesia was induced by sodium pentobarbital (30 mg/kg i.v.) and maintained by infusion (6 mg/kg/hr) into a catheterized jugular vein for the entire experiment with small adjustments made depending on the depth of anesthesia. The left carotid artery and jugular vein were cannulated with PE-50 tubing. Mean arterial blood pressure (MAP) was monitored with a Statham P23AA pressure transducer (Gould-Statham, Oxnard, California) attached to the catheter in the carotid artery; the jugular vein catheter was for intravenous administration of drugs. Three milliliters of blood from the left carotid artery was withdrawn for determination of the hematocrit and control plasma renin activity. Rabbits, lying either on their back (femoral experiment) or right side (renal experiment), were allowed to respire spontaneously through a cannula in the trachea. In the renal experimental group (group 1), the left renal artery and vein were exposed through a retroperitoneal flank incision. RBF was monitored with a blood flow probe (5 mm in circumference, Carolina Medical Electronics, King, North Carolina) placed on the renal artery and connected to a Carolina electromagnetic flowmeter. A 25 gauge needle attached to PE-50 tubing was inserted into the renal vein for collection of renal venous samples and reinjection of the blood cells suspended in normal saline after separation of plasma by centrifugation at 4°C. In the femoral experiment (group 2), the right femoral artery and vein were exposed in the inguinal triangle. The blood flow probe (5 mm in circumference, Carolina Medical Electronics) was placed on the femoral artery for FBF recording and a 25 gauge needle inserted into the femoral vein for venous sampling, as in the renal experiment. MAP and FBF or RBF were recorded on a polygraph (Grass Instrs., Quincy, Massachusetts). In the nephrectomy experiment (group 3), the set-up was similar to that in group 2 except that the rabbits underwent bilateral nephrectomy 48 hours before the experiment to eliminate the influence of the systemic renin-angiotensin system. The operation was carried out under aseptic conditions while the rabbits were anesthetized with 30 mg/kg i.v. sodium pentobarbital. To maintain normal electrolyte balance and prevent uremia, peritoneal dialysis was performed twice a day with Dianal PD-2 (Travenol Labs. Inc., Deerfield, Illinois) during the 48 hours before the experiment. Blood samples for plasma renin activity determination were taken before and 48 hours after nephrectomy. Radioimmunoassay for plasma renin activity was conducted as described previously. For angiotensin determinations, 4 ml arterial and renal or femoral venous blood samples were collected with chilled plastic syringes containing 0.2 ml of a solution of 6 mg/ml o-phenanthroline and 40 mg/ml disodium EDTA and then centrifuged immediately. The plasma was kept frozen at -20°C before analysis of Ang I and Ang II. Ang I and Ang II were extracted on a Dowex 50-X2 column using a modification of a previously published method, and the eluate was evaporated to dryness and reconstituted for radioimmunoassay. Antisera for both Ang I and Ang II were used. The Ang I antiserum did not cross-react with Ang II, 10–500 pg/ml plasma. Cross-reactivity of the Ang II antiserum with Ang I, 1.25–50 ng/ml plasma, was 0.1–0.2%. Cross-reactivity of the Ang II antiserum was 100% for the Ang-(2–8) heptapeptide. Because of the cross-reactivity of the Ang II antiserum, values of plasma Ang II concentration represent immunoreactive Ang II and are not meant to equal the absolute concentration. The Ang I values are presumed to be more accurate quantitatively.

**Experimental Protocol**

Approximately 15 minutes were allowed for the MAP and RBF to stabilize after completion of the surgical procedure, and then a second blood sample for plasma renin activity was taken. Ang I (US Biochemicals, Cleveland, Ohio) was then injected intravenously (0.15 µg/kg) into the jugular vein to elicit control arterial pressure and blood flow responses. No Ang I was administered in the nephrectomized rabbits. The maximal changes in MAP and RBF or FBF were the values taken for analysis. Thirty minutes later, control arterial (A1) and renal or femoral venous (V1) samples were taken for angiotensin determination. Ten minutes after the sampling, captopril (The Squibb Institute for Medical Research, Princeton, New Jersey) was administered in a bolus dose of 2 mg/kg i.v., and a continuous infusion of 1 mg/kg/hr was started and given for the remainder of the experiment to block the systemic and local renin-angiotensin systems. At 10 minutes after captopril, A2 and V2 samples were taken, and the dose of Ang I was injected at 20 minutes after captopril to indicate the degree of blockade of the Ang I response. The final samples of A3 and V3 were taken at 70 minutes and the dose of Ang I repeated at 80 minutes after captopril. After the withdrawal of each pair of blood samples and centrifugation, the resuspended blood cells were returned to the rabbit.

To exclude the possible influence of the injected Ang I on plasma Ang I levels, three additional groups of experiments (groups 4–6) were conducted to analyze the effect of captopril on plasma Ang I concentration. Arterial and renal or femoral venous samples before and after captopril were collected at the same intervals as those described above. In group 4 (n=6), only a bolus injection of captopril (2 mg/kg i.v.) and a continuous infusion (1 mg/kg/hr i.v.) were administered after taking control arterial and renal venous samples. In group 5 (n=5), saline instead of captopril was injected (2 ml i.v.) and infused (0.1 ml/min i.v.) after the control samples were taken. Group 6 served as a time control for changes in Ang I levels due to the experimental procedure itself. In group 6 (n=4), the same dose of captopril was administered, and the arterial and femoral venous samples were collected at the same intervals as those in all other groups. To prevent the in vitro production of Ang I from angiotensinogen during sampling,
pepstatin A (Bachem Feinchemikalien AG, Switzerland), a potent renin inhibitor, was added to the cocktail in the syringes equivalent to a final concentration of 10 μM in the blood samples of these groups. Sampling volume was reduced to 2 ml/sample to minimize the influence of blood withdrawal on the Ang I levels. The Ang I was assayed directly in the plasma without extraction.

Statistical Analysis

Data are expressed as mean±SEM. Statistical significance was determined by one-way analysis of variance (ANOVA) with repeated measures, two-factor ANOVA with one repeated measure and by paired t test where appropriate. A p value of less than 0.05 was considered significant. The Scheffe procedure was used to test for significant differences between means after ANOVA.

Results

Plasma Renin Activity

The control values of plasma renin activity were 2.4±0.2 and 3.0±0.6 ng Ang I/ml/hr in group 1 (renal experiment) and group 2 (femoral experiment), respectively; after completion of the surgical procedure, the values increased to 7.5±1.0 (p<0.05) and 5.0±1.2 ng Ang I ml/hr (NS). In group 3 (nephrectomy experiment), the plasma renin activity value was 2.47±0.16 ng Ang I/ml/hr before nephrectomy. On the day of the acute experiment, 48 hours after the operation, the plasma renin activity decreased to 0.07±0.03 ng Ang I/ml/hr, which indicates effective elimination of the systemic renin-angiotensin system. After the surgery, plasma renin activity increased slightly to 0.24±0.05 ng Ang I/ml/hr.

In groups 4, 5, and 6, the plasma renin activity values were 2.8±0.8, 3.2±0.8, and 2.3±0.5 ng Ang I/ml/hr before the surgical procedure and 5.1±1.0 (p<0.05), 5.8±1.9 (p<0.05), and 1.9±0.5 (NS) after completion of the surgical procedure, respectively. The retroperitoneal flank incision to expose the kidney results in a higher plasma renin activity.

Hemodynamic Effect

Figure 1 shows the effects of captopril on the hemodynamic response to intravenous injection of Ang I in groups 1 and 2. Ang I (0.15 μg/kg i.v.) increased MAP by 18±1 mm Hg in group 1 and by 19±1 mm Hg in group 2 (Figure 1A). It decreased RBF by 35±3% but increased FBF by 31±8% (Figure 1B), thus increasing renal vascular resistance (RVR=MAP/RBF) by 87±10% (Figure 1C) without having a significant effect on femoral vascular resistance (FVR=MAP/FBF). At both time periods after its administration, captopril blocked the response to Ang I in the two groups.

The hemodynamic response to captopril itself differed between the renal and femoral experiments (Table 1). Captopril caused a similar decrease in MAP in both groups of experiments; however, it increased RBF by 32±10% (10-minute value) and decreased FBF by 21±4%. As a result, RVR was decreased 36±5% by captopril, but FVR was unchanged. At 70 minutes after captopril, MAP was

<table>
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<th>Table 1. Effects of Captopril on Systemic Blood Pressure, Renal and Femoral Blood Flow, and Vascular Resistance</th>
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<tr>
<td>MAP (mm Hg)</td>
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<td>Blood flow (ml/min)</td>
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<td>Vascular resistance (mm Hg/ml/min)</td>
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Values are mean±SEM. Δ, change of values after captopril at time indicated; MAP, mean arterial blood pressure. *p<0.05 versus control.
**TABLE 2.** Effect of Captopril on Systemic Blood Pressure, Femoral Blood Flow and Vascular Resistance, and Arterial and Femoral Venous Angiotensin II in Group 3 Nephrectomized Rabbits

<table>
<thead>
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<th>Variables</th>
<th>Control</th>
<th>10 min</th>
<th>70 min</th>
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<tr>
<td>MAP (mm Hg)</td>
<td>74±4</td>
<td>73±4</td>
<td>69±4</td>
</tr>
<tr>
<td>FBF (ml/min)</td>
<td>11±3</td>
<td>10±3</td>
<td>10±2</td>
</tr>
<tr>
<td>FVR (mm Hg/ml/min)</td>
<td>10±2</td>
<td>10±2</td>
<td>10±2</td>
</tr>
<tr>
<td>Arterial angiotensin II (pg/ml)</td>
<td>61±10</td>
<td>50±8</td>
<td>47±8</td>
</tr>
<tr>
<td>Femoral venous angiotensin II (pg/ml)</td>
<td>51±10</td>
<td>53±10</td>
<td>45±18</td>
</tr>
</tbody>
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*Postcaptopril values are not significant versus control. Values are mean±SEM. MAP, mean arterial blood pressure; FBF, femoral blood flow; FVR, femoral vascular resistance.

Further decreased in both groups. There remained a decrease in RVR, whereas RBF fell because of the reduction in blood pressure. FVR and FBF were variably affected at the 70-minute interval.

Postnephrectomy values of MAP, FBF, and VPR before and after captopril are shown in Table 2. MAP was lower in group 3 than groups 1 or 2, but FBF and FVR did not differ from group 2. Captopril had no effect on any of these parameters in the nephrectomized rabbits.

**Plasma Angiotensin I Concentration**

The left panel of Figure 2 depicts the effect of the acute administration of captopril on arterial and renal venous Ang I levels in group 4. The control arterial and renal venous Ang I was 261±47 and 314±72 pg/ml, respectively, which were significantly increased to 1,308±264 and 2,016±441 pg/ml at 10 minutes and to 2,625±342 and 3,663±479 pg/ml at 70 minutes after captopril, respectively. The venous-arterial (V-A) concentration difference of Ang I was increased by captopril (p<0.05 by two-factor ANOVA) in this group. Similar captopril-induced increased levels of Ang I and V-A difference of Ang I were found in group 1 (Figure 3, left panel), although the absolute values of Ang I were much higher probably because of in vitro production of Ang I. In group 6, captopril also increased arterial and femoral venous Ang I levels from 192±25 and 189±25 pg/ml to 523±159 and 534±150 pg/ml at 10 minutes and to 1,391±588 and 1,491±607 pg/ml at 70 minutes after its administration, respectively, but the V-A difference of Ang I remained unchanged (Figure 2, right panel). The Ang I values in group 2 exhibited the same tendency (Figure 3, right panel). Inasmuch as RBF increased and FBF decreased after captopril, the renal Ang I secretion rate, (V<sub>AngI</sub>-<sub>A</sub><sub>AngI</sub>×RBF, would be greatly increased compared with the femoral Ang I secretion rate, (V<sub>AngI</sub>-<sub>A</sub><sub>AngI</sub>×FBF). In group 5 (saline control), the arterial and renal venous Ang I levels were slightly increased only at 70 minutes after saline infusion, suggesting a mild elevation of the Ang I level with time under the present experimental conditions.

**Plasma Angiotensin II Concentration**

Figure 4 shows Ang II levels before and after captopril in groups 1 and 2. The control arterial and renal venous Ang II was 261±47 and 314±72 pg/ml (NS) in group 1, and the control arterial and femoral venous Ang II was 98±20 and 69±12 pg/ml (p<0.05), respectively. Captopril decreased both arterial and renal or femoral venous Ang II in the two groups; however, it reduced the arterial Ang II more than the venous Ang II (~90±23 pg/ml vs. ~47±17 pg/ml at 10 minutes after captopril in group 1, p<0.05 and ~75±20 pg/ml vs. ~37±13 pg/ml at 10 minutes after captopril in group 2, p<0.05). A low

**Figure 2.** Bar graph showing effect of captopril on arterial (A) and renal or femoral venous (V) plasma angiotensin I (AI) levels in groups 1 (renal experiment only), 5 (renal experiment with saline control), and 6 (femoral experiment with captopril only), in which no angiotensin I was administered during experiment and pepstatin A was added to cocktail in syringes to prevent in vitro generation of angiotensin I. Results are mean±SEM. *p<0.05 versus control.

**Figure 3.** Bar graph showing effect of captopril on arterial (A) and renal or femoral venous (V) plasma angiotensin I (AI) levels in groups 1 and 2. Angiotensin I determinations were made in five of the 11 experiments of group 1. Results are mean±SEM. *p<0.05 versus control.
level of arterial and femoral venous Ang II remained after nephrectomy (Table 2). Captopril did not reduce the Ang II level in the nephrectomized rabbits.

Discussion

Many investigations demonstrating the presence of a tissue renin-angiotensin system have relied on the use of cultured cells, isolated perfused vascular beds, and isolated blood vessels. Further evaluation of this system in the intact rabbit seemed necessary. Thus, we sought evidence for the local generation of Ang I and Ang II and examined the influence that tissue Ang II might have on naturally perfused renal and femoral vascular beds of the anesthetized rabbit.

With this preparation, the arterial concentration of the angiotensins reflects the overall circulating level to which all vascular beds are exposed. Femoral and renal venous Ang II and Ang I are considered to represent almost totally angiotensins formed in these vascular beds. We have arrived at this assumption because of the well-documented, marked degree of catabolism of Ang II in these beds so that arterial Ang II should contribute negligibly to that emanating from the venous side. Approximately 90% and 60–90% of the Ang II presented to the renal and femoral vascular beds, respectively, is metabolized, which could not be discerned from Ang II in our radioimmunoassay, the change in immunoreactive Ang II after ACE inhibition is most important. The decrease in renal and femoral venous Ang II after captopril is an indicator of locally produced Ang II. The lower control femoral than renal venous Ang II concentration (69 vs. 110 pg/ml) suggests less local generation in the femoral bed, or possibly greater degradation, although based on a similar or even lesser degree of catabolism than in the renal bed,11 the latter possibility is less likely. Intravenously administered captopril decreased both arterial and the renal and femoral venous Ang II; however, the venous Ang II was slightly less affected. Based on these results, angiotensin converting enzyme inhibition reduces circulating Ang II and that formed in the renal and femoral vascular beds. The remaining low level of arterial and venous Ang II may represent uninhibited Ang II production, Ang II metabolites, or in vitro-generated Ang II. In the isolated perfused hind leg preparation of the rat, captopril depressed locally generated Ang II; however, the more lipophilic angiotensin converting enzyme inhibitor SA446 when administered for 1 week was more effective than captopril in reducing the Ang II released in this preparation.11 If in the present study, the remaining immunoreactive Ang II truly represents uninhibited Ang II production, it may take long-term administration of an angiotensin converting enzyme inhibitor to further decrease or eliminate this plasma Ang II.

The acute fall in MAP and increase in RBF caused by captopril correlated with the suppression of the plasma levels of Ang II, indicating that under these experimental conditions Ang II supported blood pressure and maintained renal vascular tone. Whether this relates to the influence of circulating or locally produced Ang II or to the effect of both is a matter of conjecture. Administration of Ang I intravenously also had a marked vasoconstrictor effect on the renal vasculature. In contrast to the renal vascular bed, the femoral vasculature was insensitive to both exogenously administered Ang I and to endogenous Ang II. There was no change in FVR due to the administration of Ang I intravenously or after angiotensin converting enzyme inhibition with captopril. These results reflect the relatively greater difference in sensitivity to the vasoconstrictor action of Ang II between the renal and femoral vascular beds.

As would be expected, captopril caused an increase in plasma Ang I, which is attributable to blockade of the Ang II–mediated negative feedback of renin release. A striking difference was noted, however, in the renal and femoral venous levels of Ang I. In both vascular beds, the Ang I levels were similar in the arterial and venous plasma before captopril; however, a positive V–A difference of Ang I developed in the renal but not in the femoral bed after angiotensin converting enzyme inhibition. Elimination of the negative feedback of renin release in the renal bed allows expression of increased intrarenal production of Ang I. However, there was no evidence of a similar phenomenon in the femoral vasculature. Thus, after angiotensin converting enzyme inhibition the differential ability of the kidney and femoral vascular bed to generate angiotensin can be demonstrated.

Because the FBF is approximately 25% of that of the RBF, it is conceivable that greater metabolism may occur in the femoral bed to contribute to a lower venous Ang I. Arguing against this possibility is the similarity of the control femoral and renal venous Ang I levels. In group 2, the control femoral venous Ang I was slightly higher and in group 6 slightly lower than the control renal venous Ang I in groups 1 and 4, respectively.
Because nephrectomy has been reported to increase aortic messenger RNA for angiotensinogen and presumably to increase activity of the tissue renin-angiotensin system, we used this approach to stimulate the femoral renin-angiotensin system. In group 3, nephrectomy markedly reduced the plasma renin activity, but as was seen after angiotensin converting enzyme inhibition, there remained a low level of arterial and femoral venous Ang II. Earlier reports have also documented persistence of Ang II in nephrectomized humans and animals. The residual plasma Ang II or Ang II metabolites may be derived from the tissue renin-angiotensin system; however, high-performance liquid chromatography is required for characterization of this immunoreactive Ang II. Acute administration of captopril had no effect on the arterial or femoral venous Ang II level, nor did it alter blood pressure or FVR in group 3. These results fail to provide evidence for a greater local hind limb generation of Ang II after nephrectomy, and they don’t suggest a functional role of locally formed Ang II in the maintenance of hind limb vascular tone after eliminating the renal renin-angiotensin system.

The present findings reveal that the rabbit renal vascular bed is more sensitive to endogenously formed Ang II and has a greater capacity to generate Ang II than the femoral bed. A decrease in renal and femoral venous Ang II after the administration of captopril suggests the local production of Ang II by these vascular beds. The relative role of circulating and locally produced Ang II in influencing RBF in the anesthetized rabbit is yet to be defined.

Acknowledgment

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References


Key Words: renin-angiotensin system • blood flow • nephrectomy • kidney • converting enzyme inhibition
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