Regulation of Vascular Angiotensin Release
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To investigate the regulatory mechanism of the vascular renin-angiotensin system, we perfused isolated rat hind legs with plasma-free buffer and quantified angiotensin peptides in the perfusate. Angiotensin release from hind legs was increased in rats pretreated with losartan (DuP 753) and rats fed a low sodium diet with subsequent furosemide and was decreased in nephrectomized rats and rats given dexamethasone, ethynylestradiol, and triiodothyronine. Using these models, we have attempted to identify which step or component of angiotensin metabolism determines angiotensin release level. Changes caused by these manipulations in plasma renin concentration and basal angiotensin release from hind legs were almost parallel, whereas plasma angiotensinogen concentration and the angiotensin release changed in opposite directions. Infusion of renin in hind legs caused a marked increase in angiotensin release and continued even 1 hour after cessation of renin infusion. Infusion of angiotensinogen did not alter the angiotensin release. Angiotensin clearance and angiotensin I conversion were not affected by either nephrectomy or losartan pretreatment. Aortic renin messenger RNA level was extremely low and not increased by nephrectomy or losartan pretreatment, although kidney renin messenger RNA level was increased by losartan pretreatment. These results provide evidence that plasma renin of kidney origin is the major source of vascular functional renin and plays the determining role in the regulation of vascular angiotensin release. Plasma-derived or locally produced angiotensinogen, locally produced renin, converting enzyme, and angiotensin clearance are not considered to be the primary determinant in the regulation of vascular angiotensin release in these acute and subacute experimental models. (Hypertension 1993;21:446-454)

KEY WORDS • angiotensin release • vascular renin-angiotensin system • losartan • perfusion

The renin-angiotensin system plays important roles in blood pressure regulation and fluid and electrolyte homeostasis. Large numbers of studies have suggested that angiotensins are produced not only in circulation but also in local tissues, including kidney, adrenal, brain, and peripheral vasculature. A high clearance rate of angiotensins in vasculature and the relatively small amount of angiotensin generation by plasma renin has led to the view that substantial angiotensin generation should occur in the vasculature. We have directly demonstrated local angiotensin generation in isolated perfused mesenteric artery, hind leg, and kidney.

Although local generation of angiotensin has been reported, it still remains unclear whether angiotensin is produced by locally synthesized components or by plasma-borne components of the renin-angiotensin system, in other words, whether local angiotensin production is totally independent of the circulating renin-angiotensin system. Swales et al demonstrated arterial wall uptake of renin, and we have reported the importance of vascular renin of renal origin in maintaining hypertension of spontaneously hypertensive rats. Using these models, we have attempted to identify which step or component of angiotensin metabolism determines angiotensin release level. Changes caused by these manipulations in plasma renin concentration and basal angiotensin release from hind legs were almost parallel, whereas plasma angiotensinogen concentration and the angiotensin release changed in opposite directions. Infusion of renin in hind legs caused a marked increase in angiotensin release and continued even 1 hour after cessation of renin infusion. Infusion of angiotensinogen did not alter the angiotensin release. Angiotensin clearance and angiotensin I conversion were not affected by either nephrectomy or losartan pretreatment. Aortic renin messenger RNA level was extremely low and not increased by nephrectomy or losartan pretreatment, although kidney renin messenger RNA level was increased by losartan pretreatment. These results provide evidence that plasma renin of kidney origin is the major source of vascular functional renin and plays the determining role in the regulation of vascular angiotensin release. Plasma-derived or locally produced angiotensinogen, locally produced renin, converting enzyme, and angiotensin clearance are not considered to be the primary determinant in the regulation of vascular angiotensin release in these acute and subacute experimental models. (Hypertension 1993;21:446-454)

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Methods

Animals

Male Sprague-Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, Ind.) weighing 350–400 g were used in three groups: normal control rats, rats bilaterally nephrectomized 36 hours before perfusion, and rats given the Ang II subtype I receptor antagonist losartan (DuP 753, 20 mg/kg, provided by Du Pont Merck Pharmaceutical Co., Wilmington, Del.) for 1 week in drinking water. Additional experiments used rats prepared in the following manners: nephrectomized rats with subsequent subcutaneous injection of losartan (10 mg/kg) every 12 hours for 36 hours before perfusion; intact rats treated with subcutaneous dexamethasone (7 mg/kg), 17a-ethynylestradiol (3 mg/kg), and 3,3',5-triiodothyronine (40 /ug/kg) for 2 days before perfusion (Dex+E2+T3); and rats fed a low sodium diet (NaCl 0.03%, Ralston Purina, St. Louis, Mo.) for 1 month with subsequent subcutaneous injection of furosemide (20 mg/kg) twice a day for 2 days before perfusion (low sodium+furosemide).

Isolated Perfused Hind Legs

Preparation. We modified our previous methods mainly according to the method of Hilgers et al. to minimize both the edema of preparation and leakage of perfusate. Briefly, rats were anesthetized with pentobarbital sodium (40 mg/kg i.p.). After median laparotomy and heparinization (1,300 IU/kg i.v.), testes and gut were excised and a cannula (PE-90) was inserted in the abdominal aorta and a second cannula (PE-205) in the inferior vena cava; the animal then was transected at the level of renal pedicles. The bilateral hind legs were thoroughly flushed by Krebs-Ringer solution (millimolar concentrations: NaCl 112, KCl 5.0, NaH2PO4 1.0, MgSO41.2, CaCl2 2.5, NaHCO3 25, and glucose 11.2) to minimize both the edema of preparation and leakage of perfusate. Briefly, rats were anesthetized with pentobarbital sodium (40 mg/kg i.p.). After median laparotomy and heparinization (1,300 IU/kg i.v.), testes and gut were excised and a cannula (PE-90) was inserted in the abdominal aorta and a second cannula (PE-205) in the inferior vena cava; the animal then was transected at the level of renal pedicles. The bilateral hind legs were thoroughly flushed by Krebs-Ringer solution (millimolar concentrations: NaCl 112, KCl 5.0, NaH2PO4 1.0, MgSO41.2, CaCl2 2.5, NaHCO3 25, and glucose 11.2) to eliminate blood and were placed in a water-jacketed container maintained at 37°C and perfused with Krebs-Ringer solution containing artificial colloid Ficoll 70 (Pharmacia LKB Biotechnology, Uppsala, Sweden). The 2.5% Ficoll 70 was effective in suppressing edema yet permitted adsorption of Ang II to a Sep-Pak C18 cartridge as described previously. The solution was maintained at 38°C and gassed with a mixture of 95% oxygen and 5% carbon dioxide to obtain a pH of 7.4. The hind legs were perfused at a constant flow rate of 4 mL/min with a roller pump (Polystaltic Pump, Buchler Instruments, Fort Lee, N.J.), and perfusion pressure was monitored by a pressure transducer connected to a polygraph. Protocols were started after 60 minutes of equilibration perfusion.

Basal angiotensin release. Angiotensins that were released from isolated perfused hind legs were trapped by Sep-Pak C8 cartridges as described previously. The cartridges were connected directly to the vena cava cannula for 30 minutes, during which 120 mL perfusate passed through the cartridge. Trapped peptides were eluted with 3 mL methanol/water/trifluoroacetic acid (80:19.9:0.1). The eluate was dried in a vacuum centrifuge. Angiotensins then were separated by reversed-phase high performance liquid chromatography (HPLC) as described previously. In brief, samples were loaded on a Nova Pak C8 column (0.39x15 cm, Waters Associates) and eluted with an exponential gradient (gradient No. 7 by Waters automated gradient controller model 680) of acetonitrile from 10.5% to 34.5% in 25 mM sodium phosphate buffer, pH 7.6, over a period of 20 minutes at a flow rate of 1 mL/min, and 400-µL fractions were collected and dried in a vacuum centrifuge. Samples were redissolved in 0.1 M Tris buffer, pH 7.4, and subjected to radioimmunoassay (RIA) for Ang I and Ang II. The Ang II antiserum showed less than 1% cross-reactivity with Ang I but 100% with angiotensin III [Ang III; Ang-(2-8) heptapeptide], Ang-(3-8) hexapeptide, and Ang-(4-8) pentapeptide. The sensitivity of detection of Ang I and Ang II was 1 pg per tube. The percent recoveries of Ang I and Ang II from the perfusate by extraction and HPLC were 61% and 70%, respectively, as examined with 100 pg synthetic Ang I or Ang II (Peninsula Laboratories, Belmont, Calif.) dissolved in 120 mL of the perfusion buffer. Values were not adjusted based on these percentages of recovery.

Infusion of renin and angiotensinogen. Mouse submandibular renin, prepared as described previously, was infused in hind legs from normal, nephrectomized, and losartan-treated rats at concentrations of 2 and 10 milliunits/mL in perfusate for 30 minutes with an infusion pump (Harvard Apparatus, South Natick, Mass.). Recombinant rat angiotensinogen, prepared as described previously, was infused in normal and losartan-treated rat hind legs at a concentration of 300 ng Ang I per milliliter in perfusate for 30 minutes with an infusion pump.

Angiotensin metabolism. Angiotensin metabolism was investigated largely according to the method of Hilgers et al. Briefly, for the estimation of Ang I clearance in hind leg vasculature, Ang I was continuously infused into hind legs at a concentration of 1 nM in the presence of captopril (1 µM). After hind leg perfusate was collected, the hind leg preparation was removed and control perfusate that had not been passed through the hind legs was collected. Percent clearance of Ang I was calculated as [(1—(Ang I in hind leg perfusate/Ang I in control perfusate))] x 100. Ang II clearance was measured by the same method; Ang II (0.5 nM) was continuously infused without captopril, and perfusate from hind legs and control perfusate (without being passed through hind legs) were collected and the ratio of Ang II was calculated. For the assessment of Ang I conversion to Ang II in hind legs, Ang I was continuously infused without captopril, and Ang I and Ang II in the perfusate were measured. Normal, nephrectomized, and losartan-treated rats were used in these experiments. After a 15-minute infusion of angiotensin peptides for equilibration, perfusates were collected on ice in the presence of 6.25 mM EDTA and 1.25 mM 1,10-phenanthroline. Samples were directly assayed by RIA for Ang I and Ang II as described above, and randomly selected samples were assayed by HPLC and RIA to monitor the amount of Ang II metabolites that are not distinguished from Ang II by direct RIA.

Measurement of Plasma Renin Concentration and Plasma Angiotensinogen Concentration

Blood samples for the measurement of plasma renin concentration (PRC) and plasma angiotensinogen concentration were collected on EDTA from randomly selected rats (normal, nephrectomized, losartan-treated,
Identification and Measurement of Angiotensinogen in Perfusate

Perfusate was concentrated 2.5-fold with a Centri-con-30 (Amicon, Beverly, Mass.), and the sample (50 μL) was applied to 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The electrophoresed proteins were transferred to a nitrocellulose filter and were immunostained with rat angiotensinogen antibodies and iodine-125-labeled goat anti-rabbit IgG as described previously. The rat angiotensinogen antibodies were raised in a rabbit immunized with synthetic hapten antigen, Ala$_{43}$-Ser$_{45}$-Tyr-Cys, in the carboxy terminal region of rat angiotensinogen. Its specificity to angiotensinogen was confirmed.

Angiotensinogen concentration in the perfusate was assayed by the same method as plasma angiotensinogen.

Renin mRNA Measurement by Polymerase Chain Reaction Method

As renin mRNA level in vasculature was reported to be very low, we used the polymerase chain reaction method to assess renin mRNA levels in kidney and aorta. The precise method and the validity for the measurement of renin mRNA were described in detail elsewhere. Briefly, the region between Nae I (810) and Apa I (919) sites of the rat renin complementary DNA (cDNA) clone pRen 44 (1,425-bp cDNA in pGEM-4; provided by K. Lynch, Department of Pharmacology, University of Virginia) was deleted, and the deletion-mutated renin RNA (DR-RNA) was synthesized by SP6 RNA polymerase. Total RNA (approximately 10–20 μg), isolated by the CsCl-guanidinium thiocyanate method from aortas (normal, nephrectomized, losartan-treated, and nephrectomy+losartan-treated rats) or kidneys (normal and losartan-treated rats), and DR-RNA (1 fg to 5 pg) were mixed and reverse transcribed using random primers as a primer. The resultant single-strand cDNA mixture was amplified by polymerase chain reaction using Taq DNA polymerase (Stratagene, Inc., La Jolla, Calif.). Denaturing, annealing, and polymerase reactions were done 32 cycles for kidney and 42 cycles for aorta at 95°C for 30 seconds, 59°C for 60 seconds, and 73°C for 120 seconds, respectively. The sense primer 1 used was 5'CTGG-GAGGCAGTGACCTCAACATTCCAGG-3' (747–776) and the antisense primer 2 was 5'-GAGAGCCAG-TATGCACAGGTCATCGTTCCT-3' (1,118–1,089). Amplification of rat renin mRNA and DR-RNA using these two primers should give 372-bp and 263-bp fragments, respectively. The trace amount of [α-32P]deoxyctydine 5'-triphosphate was included in the amplification mixture to measure the ratio of the amount of the 372-bp fragment to that of the 263-bp fragment by 5% polyacrylamide gel electrophoresis followed by autoradiography.

Results

Isolated Perfused Hind Legs

Basal hind legs perfusion pressures were not significantly different among the three groups (normal, 28.6±1.5 mm Hg; nephrectomy, 27.9±2.1 mm Hg; losartan treatment, 28.9±1.9 mm Hg).

Basal angiotensin release. Separation of angiotensins by HPLC is shown in Figure 1. The top panel shows separation of the mixture of synthetic peptides [Ang I, Ang II, Ang III, Ang-(3–8) hexapeptide, and Ang-(4–8) pentapeptide] detected by absorbance at 214 nm. The bottom panel shows a typical result of HPLC and RIA for angiotensin peptides from a sample extracted from

![Figure 1](http://hyper.ahajournals.org/)}
perfusate of normal rat hind legs. Four angiotensin peptides were identified by RIA for Ang II, but the amount of Ang III, Ang-(3-8) hexapeptide, or Ang-(4-8) pentapeptide was always less than 15% of that of Ang II in any experimental models examined.

As shown in Figure 2A, basal release of angiotensins from normal rat hind legs was 59.4±8.7 pg for Ang I and 115.1±17.5 pg for Ang II during a perfusion period of 30 minutes. The basal release was significantly decreased in nephrectomized rat hind legs (Ang I, 7.1±2.9 pg; Ang II, 10.3±2.9 pg) and increased in losartan-treated rats (Ang I, 237.8±27.0 pg; Ang II, 391.0±73.2 pg). Angiotensin release was also significantly, but to lesser degrees, decreased in Dex+E2+T3-treated rats (Ang I, 21.1±5.7 pg; Ang II, 24.6±4.7 pg) and increased in low sodium+furosemide-treated rats (Ang I, 124.4±15.7 pg; Ang II, 224.6±24.7 pg). We therefore selected nephrectomized and losartan-treated rats for further investigation. In addition, there was no difference in angiotensin release between nephrectomized rats and nephrectomy+losartan-treated rats (Ang I, 9.1±4.2 pg; Ang II, 15.3±2.2 pg). There was also no difference between normal rats and normal rats perfused with 1 μM losartan (Ang I, 67.2±15.3 pg; Ang II, 98.2±19.8 pg). These data suggested that the effect of losartan on angiotensin release was not its direct effect on hind leg vasculature but an indirect one mediated by the kidney.

Response to renin and angiotensinogen infusion. Exogenously infused renin induced as much as a 100-fold increase in angiotensin release from normal rat hind legs (Figure 3). In nephrectomized and losartan-treated rats, we also observed marked increases. The amounts of released angiotensin induced by renin infusion at a dose of 2 milliunits/mL were not different among normal, nephrectomized, and losartan-treated rats in spite of the large difference in basal angiotensin release. At a dose of 10 milliunits/mL, angiotensin release from nephrectomized rats was even greater than from losartan-treated or normal rats, a situation opposite of perfusion without exogenously added renin. Increase in Ang II release from rat hind legs induced by renin infusion continued even after cessation of renin infusion. Ang II release during the first and second 30 minutes after cessation of renin infusion was 115.7±4.7% and 85.6±5.1% of Ang II release during renin infusion. No change was observed in perfusion pressure in any renin infusion experiments.

Angiotensinogen infusion (300 ng Ang I per milliliter in perfusate) did not alter angiotensin release from hind legs of normal rats, low sodium+furosemide–treated rats, or losartan-treated rats (Table 1).

Angiotensin I and angiotensin II clearance. Clearance of exogenously infused Ang I was not significantly different among normal, nephrectomized, and losartan-treated rats. There was also no difference in Ang II clearance among these groups (Table 2). Clearance of both Ang I and Ang II in nephrectomized rats seemed slightly decreased although not statistically significant. Clearance of Ang I and Ang II was not different in each group.

Angiotensin I conversion to angiotensin II. Before measuring Ang I conversion to Ang II, we attempted to confirm that Ang I is converted to Ang II in rat hind legs by angiotensin converting enzyme. Infusion of captopril
FIGURE 3. Line graphs show release of angiotensin I (Ang I) and angiotensin II (Ang II) from hind legs of normal, nephrectomized (Nx), and losartan-treated (DuP) rats before (control) and after infusion of renin (2 and 10 milliunits/mL). n=7 in each group; *p<0.05 compared with normal.

(1 μM) almost completely suppressed Ang II release from normal rat hind legs (control period: Ang I, 60.5±14.9 pg per 30 minutes; Ang II, 102.0±19.9 pg per 30 minutes; captopril perfusion period: Ang I, 121.0±20.1 pg per 30 minutes; Ang II, 10.8±3.1 pg per 30 minutes [p<0.05 compared with control period], n=4), which indicates that angiotensin converting enzyme plays the major role in Ang I conversion in hind legs.

Ang I conversion was assessed by measuring the conversion of exogenously infused Ang I (1 nM) to Ang II. Table 2 shows that the ratio of Ang II in perfusate to Ang I in perfusate was not different among normal, nephrectomized, and losartan-treated rats. Without performing experiments under the condition of complete inhibition of Ang I and Ang II degradation, it is impossible to obtain the absolute rate of Ang I conversion to Ang II. However, the fact that Ang I and Ang II clearance was not different and the ratio of Ang II to Ang I was not different is taken to indicate that Ang I conversion to Ang II was not different among normal, nephrectomized, and losartan-treated rats. In the Ang I and Ang II clearance and Ang I conversion experiments, randomly selected samples were analyzed by HPLC and RIA, and the amount of Ang III, Ang-(3–8) hexapeptide, or Ang-(4–8) pentapeptide present in perfusate was always less than 15% of the amount of Ang II.

Plasma Renin Concentration and Plasma Angiotensinogen Concentration

PRC of each model is shown in Figure 2B. Comparison of PRC with variation in the levels of angiotensin release (Figure 2A) among the various models indicates parallelism between PRC and angiotensin release. On the contrary, the changes in plasma angiotensinogen concentration (Figure 2C) are inversely related to those of basal angiotensin release from the hind legs.

Identification and Measurement of Angiotensinogen in Perfusate

Angiotensinogen in perfusate was identified by immunoblot analysis. The immunostaining shows that perfusate gave a protein band positively stained by rat angiotensinogen antibody (Figure 4). The concentration of spontaneously released angiotensinogen in perfusate collected after a 60-minute equilibration period from rat hind legs was in normal rats, 508.2±52.7 pg Ang I per milliliter; in nephrectomized rats, 2,142.3±312.8 pg Ang I per milliliter (p<0.05 compared with normal rats); and in losartan-treated rats, 188.5±23.1 pg Ang I per milliliter (p<0.05 compared with normal rats); n=5.

Measurement of Renin mRNA

Kidney renin mRNA level was greatly increased by losartan treatment (Figure 5A). On the other hand, aortic renin mRNA level in normal rats was extremely low, and we could not observe any increase in aortic renin mRNA level by nephrectomy, losartan treatment, or nephrectomy+losartan treatment (Figure 5B).

Discussion

In the current experiments, we have demonstrated that basal angiotensin release from isolated perfused rat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ang I (pg/30 min)</th>
<th>Ang II (pg/30 min)</th>
<th>Ang I (pg/30 min)</th>
<th>Ang II (pg/30 min)</th>
</tr>
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<tbody>
<tr>
<td>Normal (n=6)</td>
<td>62.8±8.9</td>
<td>110.4±17.2</td>
<td>71.1±7.4</td>
<td>102.5±14.1</td>
</tr>
<tr>
<td>LS+F (n=4)</td>
<td>131.8±20.6</td>
<td>259.6±31.2</td>
<td>146.5±28.8</td>
<td>234.2±28.4</td>
</tr>
<tr>
<td>Losartan treated (n=4)</td>
<td>231.3±29.4</td>
<td>376.9±51.4</td>
<td>283.8±35.1</td>
<td>402.5±42.8</td>
</tr>
</tbody>
</table>

Ang I, angiotensin I; Ang II, angiotensin II; LS+F, low sodium diet with furosemide injection. After a 30-minute control period, angiotensinogen (300 ng Ang I/mL in perfusate) was infused for 30 minutes. Values are mean±SEM.
Plasma-Derived Renin

The uptake of plasma renin by the vascular wall has been reported to be important in blood pressure control in experiments with nephrectomized rats. The comparison of the results of basal angiotensin release (Figure 2A) and PRC (Figure 2B) strongly suggests that PRC is related to angiotensin release in perfused hind legs, although the changes of PRC between normal and losartan-treated rats are more exaggerated than the change of angiotensin release between them. We observed that angiotensin release from nephrectomy+losartan-treated rats was not larger than that from nephrectomized rats and that losartan perfusion alone in normal rat hind legs did not increase angiotensin release. These results suggest that the increase in angiotensin release by losartan treatment was mediated by the increase in renin of kidney origin. Exogenously infused renin induced a very large increase in angiotensin release. Levels of released Ang I and Ang II induced by 2 milliunits/mL renin infusion were practically identical among normal, nephrectomized, and losartan-treated rats. This result implies that the locally existing renin under the present experimental conditions is a limiting factor, whereas angiotensinogen and converting enzyme are relatively abundant. Therefore, the amount of renin determines the level of angiotensin release. Furthermore, the results that infusion of 10 milliunits/mL renin induced a greater increase in Ang I and Ang II release in nephrectomized rats and a lesser increase in Ang I release in losartan-treated rats in comparison with normal rats may suggest that only under renin-rich conditions does locally existing angiotensinogen become a determinant for angiotensin production rate. The fact that angiotensin release continued at a steady rate for 1 hour after cessation of renin infusion in hind legs is in agreement with the previous report by Hilgers et al in principle and seems to suggest that some infused renin does not remain in free solution in the circulation but is adsorbed to the vascular wall.

Locally Produced Renin

Local production of renin has long been discussed, however, relative contribution to local production of angiotensin by the locally synthesized renin and by renin of kidney origin (but localized in tissues) has never been determined at a steady rate for 1 hour after cessation of renin infusion in hind legs is in agreement with the previous report by Hilgers et al in principle and seems to suggest that some infused renin does not remain in free solution in the circulation but is adsorbed to the vascular wall.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ang I clearance (%)</th>
<th>Ang II clearance (%)</th>
<th>Ang II/Ang I in perfusate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=5)</td>
<td>68.5±4.2</td>
<td>67.9±4.2</td>
<td>6.76±1.1</td>
</tr>
<tr>
<td>Nephrectomized (n=5)</td>
<td>58.2±3.4</td>
<td>62.8±1.2</td>
<td>6.94±0.92</td>
</tr>
<tr>
<td>Losartan treated (n=5)</td>
<td>64.2±2.9</td>
<td>66.9±3.9</td>
<td>6.69±0.43</td>
</tr>
</tbody>
</table>

Ang I, angiotensinogen; Ang II, angiotensin II. Clearance of exogenously infused Ang I and Ang II was calculated as: Clearance (%) = (1 - angiotensin in hind leg perfusate/angiotensin in control perfusate without being passed through hind legs) x 100. Ang II/Ang I in perfusate is the ratio of Ang II to Ang I in perfusate from hind legs during continuous infusion of 1 nM Ang I. Values are mean±SEM.

hinter legs is increased in losartan-treated rats and decreased in nephrectomized rats. Thus, using these models, we have attempted to identify which step or component of the vascular renin-angiotensin system is important in determining the level of angiotensin release from peripheral vascular tissues.

Table 2. Clearance of Angiotensin and Conversion of Angiotensin I
FIGURE 4. Immunoblot analysis of angiotensinogen in perfusate. Perfusate was concentrated 2.5-fold. Fifty microliters of sample was analyzed with 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, blotted onto nitrocellulose and then immunostained.

FIGURE 5. Autoradiograms of 5% polyacrylamide gel electrophoresis of polymerase chain reaction products. Panel A: 20 µg of kidney total RNAs from normal (lanes 1-4) and losartan-treated (lanes 5-8) rats were mixed with 0.5 pg (lanes 1 and 5), 1 pg (lanes 2 and 6), 2.5 pg (lanes 3 and 6), and 5 pg (lanes 4 and 8) of deletion-mutated renin RNA. They were reverse transcribed and amplified 32 cycles by polymerase chain reaction with a trace amount of [α32P]deoxyadenosine 5'-triphosphate. Renin mRNA level was assessed by comparing the ratio of radioactivity of the upper band (372 bp, originated from renin mRNA) to the lower band (263 bp, originated from deletion-mutated renin RNA). Exposure time of autoradiography was 1 hour. This autoradiogram shows the increased expression of renin mRNA by losartan treatment. Panel B: 10 µg transfer RNA (lane 1) as negative control, 10 µg aortic total RNA from normal (lane 2), nephrectomized (lane 3), losartan-treated (lane 4), and nephrectomized and losartan-treated (lane 5) rats were mixed with 1 fg deletion-mutated renin RNA. They were reverse transcribed and amplified 42 cycles. Exposure time of autoradiography was 4 hours. This autoradiogram shows that the expression level of aortic renin mRNA is very low and not upregulated by nephrectomy or losartan treatment.

Locally Produced Angiotensinogen

Angiotensinogen has been reported to be produced in vascular tissue in a regulated manner,13,14,27 suggesting that locally produced angiotensinogen might be important in angiotensin production. Campbell and Habener13 showed an increase of angiotensinogen mRNA in the aorta of Dex+E2+T3-treated rats. Cassis et al14 reported that nephrectomy increased angiotensinogen mRNA in perivascular adipose tissue. However, we observed that angiotensin release from hind leg preparations of Dex+E2+T3-treated rats or nephrectomized rats was much less than that from hind legs of
normal rats. This result suggests that the level of local production of angiotensinogen does not determine the rate of local angiotensin production. Thus, the locally produced angiotensinogen does not seem to play the major role in the regulation of vascular angiotensin release. However, it is possible that it may contribute to the local production of angiotensin more significantly under a high renin condition.

Although we did not identify the origin (plasma-derived or locally produced) of angiotensinogen in perfusate, we demonstrated a spontaneous release of angiotensinogen from hind legs in perfusate, and the amount of angiotensinogen in perfusate was large enough to generate angiotensins from hind legs. Considering the possible importance of vascular uptake of plasma-borne renin and the existence of releasable angiotensinogen from hind legs, we submit that it is likely that a predominant amount of angiotensinogen is produced locally by an extracellular action of renin rather than by an intracellular mechanism, although a small amount may be produced by the intracellular action of endogenous vascular renin in endothelial or smooth muscle cells.

We have shown that angiotensinogen is not a primary determinant of angiotensin release from hind legs; however, we noted that the amount of locally existing angiotensinogen is important in determining angiotensin release level under a renin-rich condition. Furthermore, the greater difference in PRC between normal and losartan-treated rats compared with that in angiotensin release between them might be due to the decreased angiotensinogen in losartan-treated rats.

Angiotensin Metabolism

The clearance rate of Ang I and Ang II during their single passage through various organs has been shown to be very high.1,2,29 and angiotensin converting enzyme has been reported to be abundant on the vascular wall.30 Our present data are in agreement with these previous reports. Although it may be possible that changes in clearance or conversion affect the level of angiotensin release, the clearance of Ang I and Ang II and the conversion of Ang I were not significantly different among normal, nephrectomized, and losartan-treated rats. Therefore, the difference in angiotensin levels released in the perfusate among the various experimental models cannot be explained by angiotensin metabolism or Ang I conversion.

Although we observed no change in angiotensin clearance or Ang I conversion in this experiment, there are reports that have demonstrated the changes of angiotensin metabolism. An increase in vascular Ang I conversion in chronic renovascular hypertension31 and a decrease in vascular Ang II clearance in chronic uremia20 have been reported. Most of our experiments were short-term experiments, and this may be the reason for differences in results. Kuczer et al20 reported unchanged vascular angiotensin release despite low plasma renin in rats with chronic renal hypertension. Our results and these reports suggest that there may be differences in vascular angiotensin production and metabolism between acutely and chronically treated rats.

Taken together, the present results indicate that plasma renal renin is the major source of vascular renin and that the plasma-derived renin is the rate-limiting factor among those factors that participate in vascular angiotensin production and metabolism, at least in these acute or subacute experimental models.

There is no report in which the amount of local angiotensin production in vivo was directly measured. Instead of a direct measurement, the amount of locally produced angiotensin has been calculated from the clearance rate of radiolabeled angiotensin and the arteriovenous difference of angiotensin concentration and blood flow of local tissues. Recently, using this method, Li and Zimmerman29 have demonstrated that isoproterenol causes an increase of femoral vascular angiotensin production and that the increased production depends on the use of renal renin. Danser et al32 have reported that local tissue produces a substantial amount of angiotensin and that plasma renin activity is positively correlated with the amount of local angiotensin production calculated by this indirect method. This result is in agreement with our data obtained by direct measurement of angiotensin release in an ex vivo experiment.

Li and Zimmerman33 have reported that femoral vascular sensitivity to Ang II and angiotensin generation are smaller compared with the renal vascular bed. We also did not observe a significant change in perfusion pressure in the current experiments. Therefore, we could not emphasize the possible physiological importance of released Ang II in the regulation of vascular tone. However, in separate experiments, we have observed that hind leg vascular beds become tachyphylactic to Ang II infusion very rapidly in comparison with the in vivo blood pressure change by Ang II infusion. Therefore, it seems to be necessary to modify the perfusion system for the assessment of the role of the released Ang II in blood pressure control, although our system seems to be appropriate for the measurement of angiotensin release.

In summary, the comparison of basal angiotensin release and PRC and the effect of renin infusion on angiotensin release indicate that plasma renin of kidney origin is the major source of locally functional renin and plays the determining role in the regulation of vascular angiotensin release in these experimental models. Lack of correlation of angiotensin release from the hind leg vascular bed with plasma angiotensinogen concentration, infused angiotensinogen, vascular renin mRNA levels, and angiotensin clearance indicates that plasma-derived or locally produced angiotensinogen, locally produced renin, Ang I conversion, and angiotensin clearance are not the primary determinants in the regulation of angiotensin release from the vasculature, although angiotensinogen may play a role only in renin-rich conditions.

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