Increased Vascular Formation of Angiotensin II in One-Kidney, One Clip Hypertension

Romulo Leite and Maria Cristina O. Salgado

To assess the role of the vascular angiotensin II-generating system in one-kidney, one clip hypertension, we determined the angiotensin converting enzyme activity in plasma and vascular tissues and examined the pressor response to angiotensin II, angiotensin I, and tetradecapeptide renin substrate in isolated mesenteric arteries from one-kidney, one clip hypertensive rats 7 and 30 days after clipping the renal artery and in mesenteric arteries from age-matched normotensive rats. Angiotensin converting enzyme activity, determined in aortic and mesenteric tissues, was significantly augmented in the hypertensive (30 days after clipping) group, whereas plasma activity was normal. The vasoconstrictor responses elicited by angiotensin I and tetradecapeptide in arteries from hypertensive rats were found to be significantly potentiated 30 days after clipping, whereas the angiotensin II responses were basically unchanged. Saralasin completely blocked the vasoconstrictor responses, whereas captopril blocked only the responses to angiotensin I without affecting the responses elicited by angiotensin II and tetradecapeptide. Enalapril, an angiotensin converting enzyme inhibitor given intravenously to unanesthetized rats, significantly lowered the blood pressure of hypertensive rats. The pressor responses elicited by angiotensin II, angiotensin I, and tetradecapeptide were completely inhibited by saralasin, whereas enalapril blocked only the responses of angiotensin I but not those elicited by angiotensin II and tetradecapeptide. These results indicate that local formation of angiotensin II is increased in arteries of one-kidney, one clip hypertensive rats. The data obtained with tetradecapeptide renin substrate suggest an important role for nonrenin proteases in vascular angiotensin II formation. (Hypertension 1992;19:575–581)

Key Words • angiotensinogen • saralasin • captopril • enalapril • angiotensin converting enzyme • renal hypertension • angiotensin

The classic concept of the renin-angiotensin system (RAS) as an endocrine system, whereby angiotensin II (Ang II) is generated in the circulation and conveyed by blood to peripheral tissues, is being increasingly challenged by evidence suggesting that peripheral tissues are an important site of Ang II generation.12 This idea has gained substantial support in recent years by the demonstration that all essential components of the RAS are also present in a variety of tissues, including blood vessels, as determined by biochemical, immunohistochemical, and molecular biology techniques.3–8 Locally generated Ang II may influence vascular tone through different actions, such as a direct vasoconstrictor action, an ability to increase the noradrenergic neuroeffector function, and/or through stimulation of endothelial prostaglandin I2 biosynthesis.9,10

Evidence pointing to a pathophysiological role of a stimulated vascular RAS in hypertension has been reported by Okamura et al11 in two-kidney, one clip hypertensive rats. In this model of experimental hypertension, both vascular angiotensin converting enzyme (ACE) activity and the vasoconstrictor response to angiotensin I (Ang I) in isolated arteries were increased despite the fact that the plasma RAS was not stimulated.

One-kidney, one clip (1K1C) hypertension has been considered to be independent of the circulating RAS, with normal or low plasma levels of renin activity.12 However, the in vivo extent of Ang I conversion was found to be increased in chronic 1K1C hypertensive rats.13 In addition, Ang I elicited greater potentiation of the sympathetic nerve stimulation–induced vasoconstriction in arteries isolated from 1K1C than from normotensive rats.14 During the development of 1K1C hypertension, ACE activity measured in plasma did not change, whereas vascular ACE activity increased.14,15

The present experiments were designed to study the vascular Ang II–generating system in 1K1C hypertension by investigating the vasoconstrictor responses to Ang II, Ang I, and tetradecapeptide renin substrate (TDP) in the isolated mesenteric arteries of 1K1C hypertensive and age-matched normotensive rats. In addition, ACE activity in plasma and vascular tissues of 1K1C rats was determined, and the effect of inhibitors of RAS on the peptide pressor responses were analyzed both in vitro and in vivo.

Methods

Experiments were performed using male Wistar rats provided with standard rat chow and water ad libitum. All surgical procedures were performed on ether-anesthetized rats.

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One-Kidney, One Clip Hypertension

To induce 1K1C hypertension, a silver clip (0.25-mm i.d.) was placed around the left renal artery, and a right nephrectomy was performed after decapsulation in rats weighing 160–180 g. The 1K1C rats were used 7 and 30 days after clipping the renal artery. The day before the in vivo experiments, the rats were anesthetized and a polyethylene catheter (PE-10) (Clay-Adams, Parsippany, N.J.) was inserted into the femoral artery for direct blood pressure measurements. Blood pressure was recorded in unrestrained conscious rats with an HP-7754B recorder and an HP-1280C transducer (Hewlett-Packard, Palo Alto, Calif.) 5 hours (in vitro experiments) or 24 hours (in vivo experiments) after placing the arterial catheter. Only the animals that presented mean blood pressure (MBP) greater than 125 mm Hg on the seventh day or 150 mm Hg on the thirteenth day after clipping were used. Age-matched normotensive rats were used as a control group and were submitted to the same procedures as the 1K1C rats as described above.

Plasma and Tissue Angiotensin Converting Enzyme Activity

Blood was collected by heart puncture into a heparinized syringe while the animals were under light ether anesthesia. The superior mesenteric artery was then cannulated with a piece of PE-50 tubing, and the mesenteric bed was washed with 10 ml saline containing heparin (500 IU) and then was rapidly removed by cutting the intestinal border of the mesentery. After exsanguination, the whole aorta was removed and cleaned of adhering connective tissue and surface vessels. Blood was carefully washed from the two tissues with cold saline.

Blood samples were centrifuged for 15 minutes at 800g at room temperature, and plasma aliquots were removed and stored at −20°C until assayed. Plasma ACE activity was determined by the modified method of Friedland and Silverstein; the detailed procedure has been described by Santos et al.17 Rat plasma (10 μl) was incubated with 5 μM Hip-His-Leu in 500 μl of 0.4 M sodium borate buffer, pH 8.3, containing 0.9 M sodium chloride. The enzyme reaction was stopped by the addition of 1.2 ml of 0.34 M NaOH. The blank samples were prepared by reversing the order of addition of enzyme and NaOH. The reaction mixture was then centrifuged at 800g for 5 minutes at room temperature after the addition of 100 μl o-phthaldialdehyde in methanol (20 mg/ml), followed 10 minutes later by the addition of 200 μl of 3N HCl. The product, His-Leu, was measured fluorometrically (365 nm excitation and 495 nm emission; Aminco model 14-7561 fluoromonitor, American Instrument Co., Silver Springs, Md.). Standard curves for His-Leu (1–60 nmol) were prepared under the same conditions. All measurements were made in duplicate. The stability as well as the estimation of the effect of dipeptidase activity (less than 5%) was checked in both normal and hypertensive rat plasma. Aorta and mesentery were carefully minced into small pieces and immediately placed in an ice-cold glass homogenizer containing 10 volumes of 0.05 M sodium borate buffer (pH 7.4) containing 0.32 M sucrose. The homogenized samples were centrifuged for 10 minutes at 12,000g at 4°C, and the supernatant was stored at −20°C until assayed. The aorta and mesenteric supernatant (50 μl) were incubated with substrate as described for the plasma ACE assay. ACE activity was expressed in nanomoles His-Leu per minute per gram tissue or per milliliter plasma. The stability as well as the estimation of the effect of dipeptidase activity on the ACE activity measurements was checked in all samples from hypertensive and control rats. For a complete description of the assay, including the ACE activation by chloride, see Santos et al.17

Mesenteric Arteries Preparation

The mesenteric arterial bed of 1K1C and normotensive control rats was prepared for perfusion as described by McGregor.18 The rats were anesthetized and, after the abdominal cavity was opened, the superior mesenteric artery was perfused with 10 ml modified Krebs solution (mM: NaCl 120.0, KCl 4.7, CaCl2 3.0, MgCl2 1.43, NaHCO3 25.0, KH2PO4 1.17, glucose 11.0, and EDTA 0.03) that contained 500 IU heparin with use of a cannula (PE-50) inserted into the artery at its origin from the aorta. The cecal, ileocolic, colic, and pancreaticoduodenal branches of the superior mesenteric artery were tied off. The intestine was severed from the mesentery by cutting close to the intestinal border, and the mesentery was removed from the rat and placed ready for perfusion in a water-jacketed organ bath maintained at 37°C. The mesenteric arteries were perfused at a constant rate of 4 ml/min with Krebs solution equilibrated with 95% O2–5% CO2 at 37°C using a peristaltic pump (LKB-2115 multiperpex pump, Bromma, Sweden). Perfusion pressure was monitored via a side arm of the mesenteric artery perfusion cannula by a pressure transducer (HP-1280C, Hewlett-Packard) connected to a recorder (HP-7754A, Hewlett-Packard). After a 30-minute period of stabilization, phenylephrine was added to the perfusion solution at increasing concentrations in the range of 0.5 to 1.0 μg/ml until a stable perfusion pressure (approximately 100 mm Hg) was achieved, thus avoiding Ang II tachyphylaxis.19

Dose–response curves for Ang II (2.4–38.4 pmol), Ang I (19.3–308.8 pmol), and TDP (142.1–2273.6 pmol) were obtained by bolus injection of 10–40 μl of the peptide solution (diluted in Krebs) into the perfusion stream before the pump. Injection of the same volumes of Krebs solution did not affect the perfusion pressure. The effect of RAS inhibitors on the pressor responses to Ang II, Ang I, and TDP was tested in mesenteric arteries of normotensive and 1K1C (30 days after clipping) rats. The responses to Ang II (30 pmol), Ang I (50–200 pmol), and TDP (300–1,800 pmol) were determined before and after the addition of captopril (1–2 μg/ml) or saralasin (50 ng/ml) to the perfusion solution.

Blood Pressure Measurements

Blood pressure was recorded in conscious unstrained rats, and when blood pressure had stabilized, the effect of enalapril (2 mg/kg i.v.) or saralasin (10 μg/min/kg i.v.) on the pressor response to intravenously injected Ang II (10 pmol), Ang I (20 pmol), and TDP (60–360 pmol) was also studied in 1K1C hypertensive rats (30 days) and normotensive controls. Because of the short duration of action of a single intravenous dose
of captopril, enalapril was chosen as ACE inhibitor in the in vivo experiments.

The effect of pepstatin on the pressor response (approximately 40–50 mm Hg) elicited by angiotensinogen (250 pmol i.v.) was also examined. The dose of pepstatin used (0.5 mg/kg i.v.) completely blocked the acute renin-dependent hypertension produced by unclamping the renal pedicle after it had been occluded for 4–5 hours in conscious rats, as described elsewhere.

**Drugs, Hormones, and Reagents**

Captopril was obtained from Squibb, Princeton, N.J., and enalapril from Merck Sharp & Dohme Research Laboratories, Rahway, N.J. Pepstatin was purchased from Peninsula Laboratories Inc., Belmont, Calif. Phenylephrine, o-phthalaldehyde, and TDP (porcine) were purchased from Sigma Chemical Co., St. Louis, Mo. Ang I, Ang II, Hip-His-Leu, His-Leu, and saralasin were synthesized by Dr A.C.M. Paiva (Escola Paulista de Medicina, São Paulo, Brazil). The concentration of each peptide stock solution (1 mg/ml) was determined by amino acid analysis. The analysis of Ang I and TDP samples by reverse-phase, high-performance liquid chromatography revealed no contamination with Ang II.

**Statistical Methods**

Results are expressed as the mean±SEM. Statistical analyses were performed by the paired and unpaired Student’s t test or Scheffe’s test when analysis of variance indicated significant differences. The dose-response curves were compared by multivariate analysis of variance for repeated measurements followed by Wilks’ test. Differences were considered significant if the value of p<0.05.

**Results**

**Plasma and Tissue Angiotensin Converting Enzyme Activity**

Seven days after clipping the renal artery, MBP was significantly higher (p<0.001) in 1K1C rats (142±4 mm Hg; n = 12) than in age-matched control rats (111±3 mm Hg; n = 10). Thirty days after clipping, MBP was 182±5 mm Hg (n = 12) in 1K1C and 142±3 mm Hg in control rats (n = 9; p<0.001). ACE activity determined in plasma of 1K1C rats 7 and 30 days after clipping was similar to that of normotensive control rats (Figure 1). However, the ACE activity determined in aorta and mesentery homogenate increased during the development of hypertension (Figure 1). Aorta and mesentery ACE activities found in the 1K1C 30-day group were significantly greater (p<0.001) than those determined in the age-matched controls (367±61 versus 133±9 nmol His-Leu/min/g aortic tissue and 132±9 versus 82±5 nmol His-Leu/min/g mesenteric tissue, respectively).

**Isolated Mesenteric Arteries**

The increases in perfusion pressure elicited by different doses of Ang II, Ang I, and TDP in mesenteric arteries of 1K1C rats 7 and 30 days after clipping and age-matched normotensive rats are shown in Figure 2. No difference was found between the dose-response curves to Ang II obtained from 1K1C (n = 7, 7 days; n = 7, 30 days after clipping) and control (n = 7, 7 days; n = 13, 30 days after clipping) preparations in the 7- (p=0.183) or 30- (p=0.072) day groups. The pressor responses elicited by Ang I in the 1K1C mesenteric arteries (n = 7) were greater at 7 days (p<0.05) and presented a further increase 30 days after clipping (n = 9; p<0.01) when compared with the age-matched control groups (n = 9, 7 days; n = 13, 30 days after clipping). TDP elicited greater, but not significant, increases in perfusion pressure of 1K1C mesenteric arteries on the seventh day (n = 14) than in the controls (n = 6). Thirty days after clipping, the pressor responses elicited by TDP were significantly (p<0.01) greater in the 1K1C rats (n = 10) when compared with the controls (n = 14).

Calculated on an equipressor basis (50 mm Hg) from data presented in Figure 2, Ang I and TDP had 10.5–11.6% and 1.4–1.8%, respectively, of the pressor activity of Ang II on isolated mesenteric arteries from normotensive rats (Table 1). Both Ang I and TDP exhibited an increase in their pressor activity with the development of hypertension when compared with that of Ang II (21.6% and 2.1%, 7 days; and 35.7% and 3.1%, 30 days after clipping, respectively).

The effect of RAS inhibitors on the responses elicited by Ang II, Ang I, and TDP was examined in isolated vessels from 1K1C and normotensive rats. Saralasin (50 ng/ml) completely blocked the pressor responses (45–50 mm Hg) elicited by Ang II, Ang I, and TDP.
Captopril did not affect the vasoconstrictor response (45–50 mm Hg) elicited by Ang II. In the presence of captopril the pressor response elicited by Ang I was significantly reduced, whereas TDP responses were unaffected (Figure 3). TDP induced a longer-lasting increase in perfusion pressure when compared with that obtained with Ang I or Ang II, but both its magnitude and duration were unaffected by captopril, as shown in Figure 4.

**Blood Pressure Assay**

Intravenous injection of enalapril (2.0 mg/kg) significantly lowered the MBP of 1K1C hypertensive rats (30 days) 15 minutes after administration from 181±3 to 162±4 mm Hg (n=13; p<0.05) but did not change the MBP of normotensive control rats (from 110±1 to 105±2 mm Hg; n=11).

Saralasin (10 µg/min/kg i.v.) completely blocked the pressor responses (approximately 40–50 mm Hg) elicited by Ang II (10 pmol, n=3), Ang I (20 pmol, n=3), and TDP (240 pmol, n=3) in normotensive rats. Enalapril (2 mg/kg i.v.) abolished the pressor response to Ang I (20 pmol, n=4) without affecting the pressor response induced by Ang II (10 pmol, n=4). Enalapril administration induced a nonsignificant decrease in the amplitude of the pressor responses elicited by TDP (Figure 5) but significantly (p<0.05) diminished the duration (about 50%) of TDP pressor effect (Figure 6) in both 1K1C (30 days, n=13) and age-matched normotensive rats (n=11). Pepstatin (0.5 mg/kg i.v.) administered immediately before or after (at maximal response) the TDP injection (250 pmol i.v.) did not affect the TDP pressor responses of three normotensive rats.

**Discussion**

The 1K1C model of experimental hypertension is generally considered to be non-renin-dependent, except for the very early stages. This assumption is based on data on the temporal relations between changes in plasma renin activity and the development and maintenance of different levels of 1K1C hypertension. However, the results of the present study demonstrate that local generation of Ang II may be increased in arteries from 1K1C hypertensive rats since vascular (aorta and mesenteric bed), but not plasma, ACE activity increased during the development of hypertension. In

![Figure 2. Line plots show changes in perfusion pressure of mesenteric arterial bed isolated from one-kidney, one clip (1K1C) hypertensive rats 7 (upper panel) and 30 (lower panel) days after clipping and from age-matched normotensive controls, induced by angiotensin II (Ang II), angiotensin I (Ang I), and tetradecapeptide renin substrate (TDP). *p<0.05 and **p<0.01 (multivariate analysis of variance for repeated measurements and Wilks' method).](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>Ang II (pmol)</th>
<th>Ang I (pmol)</th>
<th>TDP (pmol)</th>
<th>Ang I/Ang II (%)</th>
<th>TDP/Ang II (%)</th>
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<td>NC</td>
<td>17±2 (7)</td>
<td>161±34 (9)</td>
<td>914±146 (6)</td>
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<tr>
<td>1K1C</td>
<td>12±2 (7)</td>
<td>56±9 (7)*</td>
<td>571±70 (14)</td>
<td>21.6</td>
<td>2.1</td>
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<tr>
<td>7 days</td>
<td></td>
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<tr>
<td>NC</td>
<td>25±4 (13)</td>
<td>214±28 (13)</td>
<td>1,748±219 (14)</td>
<td>11.6</td>
<td>1.4</td>
</tr>
<tr>
<td>1K1C</td>
<td>14±3 (7)</td>
<td>40±5 (9)†</td>
<td>462±118 (10)†</td>
<td>35.7</td>
<td>3.1</td>
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<td>30 days</td>
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Values are mean±SEM. Number of rats is shown in parentheses. Ang II, angiotensin II; Ang I, angiotensin I; TDP, tetradecapeptide; NC, normotensive control; 1K1C, one-kidney, one clip hypertension.

*p<0.05 and †p<0.01 compared with their controls, unpaired t test.
addition, an ACE inhibitor, enalapril, acutely administered intravenously to unanesthetized rats, significantly lowered the MBP of chronic 1K1C hypertensive rats. A similar dissociation of vascular and circulating RAS activities was found in long-term two-kidney, one clip11 and 1K1C14,15 hypertension.

Ang II, Ang I, and TDP produced dose-related vasoconstrictor responses in the isolated mesenteric arteries. Although we have not actually measured Ang II release, the demonstration that saralasin, an Ang II receptor antagonist, blocked the vasoconstrictor effect of Ang II, Ang I, and TDP strongly suggests that the actions of both Ang I and TDP were preceded by their conversion to Ang II in the rat mesenteric arteries.

The responsiveness of the mesenteric arteries to Ang II during the development of 1K1C hypertension was basically unaltered. Although we are not aware of any report on Ang II reactivity in vessels of 1K1C hypertension, this finding contrasts with most of the available data on vascular sensitivity in other models of experimental hypertension.22 Also, using the mesenteric arteries preparation we found that an increase in sensitivity to exogenous norepinephrine develops during 1K1C hypertension.14 Hypersensitivity in hypertension may be the result of structural changes causing an increase in the wall-to-lumen ratio or of factors beyond the vascular membrane leading to possible changes in the excitation–contraction coupling mechanism.22,23 Thus, the unchanged responsiveness to Ang II found in 1K1C vessels may reflect alterations specifically related to Ang II action, such as changes in membrane Ang II receptors. We may hypothesize that increased tissue Ang II generation in 1K1C hypertension decreases the receptor number in vascular tissue. In fact, it has been shown that during sodium depletion or Ang II infusion, vascular smooth muscle Ang II receptor number decreased (downregulated).24

Although the vasoconstrictor responses elicited by Ang II in the 1K1C preparations were similar to those obtained in the controls, the Ang I responses were significantly potentiated in the mesenteric arteries of
These findings are consistent with those reported for the rat isolated kidney \(^{25}\) and rat isolated caudal artery, \(^{26}\) mesenteric arteries from either 1K1C or control rats. Captopril did not demonstrate in our experiments. Ang II from TDP could be due to the increased ACE activity found in vascular tissue of 1K1C hypertensive rats \(^{30}\) days after clipping the mesenteric bed increased twofold and threefold \(^{7}\) and one-kidney, one-clip (1K1C) \(^{30}\) days) hypertensive rats when compared with the age-matched normotensive \(^{25}\) and rat isolated caudal artery, \(^{26}\) when given either immediately before or after administration of TDP. Since TDP response was blocked by saralasin, these data indicated that TDP is converted to Ang II directly from both Ang I and TDP into the perfusion medium, as monitored by reverse-phase chromatography. \(^{34}\) Whether this Ang II-forming enzyme found to be secreted by mesenteric arteries is the same as that responsible for TDP conversion to Ang II, described in the present study, remains to be established.

To determine the contribution of this alternative pathway, observed in vitro, to the in vivo Ang II formation, we studied the effect of ACE inhibition on blood pressure responses to TDP in conscious rats. Enalapril, at a dose that completely blocked the pressor response to Ang I, did not affect the maximal increase in blood pressure elicited by TDP, although it significantly reduced its duration. Also, pepstatin, an aspartyl protease inhibitor that competitively inhibits renin, produced no modification in the TDP pressor responses, when given either immediately before or after administration of TDP. Since TDP response was blocked by saralasin, these data indicated that TDP is converted to Ang II to a greater extent in vivo by a process that does not involve metabolism of Ang I (i.e., by a nonrenin mechanism). Assuming that endogenous angiotensinogen behaves like the TDP substrate used in this study, the hypotensive response induced by ACE inhibitor observed in 1K1C hypertensive rats cannot be due to suppression of the local formation of Ang II and may be related to accumulation of endogenous kinins that are potent vasodilator peptides. \(^{35}\)

The physiological significance of these findings is not clear since we do not know whether TDP is an effective substitute for the true renin substrate, rat angiotensinogen, or whether hydrolysis of TDP might occur at a site that is inaccessible to ACE inhibitors. To further elucidate these problems, experiments with purified rat angiotensinogen and specific protease inhibitors will be necessary.

In conclusion, the potentiated responses elicited by Ang I and TDP observed in arteries of 1K1C hypertensive rats suggest that local formation of Ang II is increased in this model of hypertension. The data obtained with TDP support this hypothesis but indicate that the major enzymatic pathway for Ang II formation is not blocked by ACE inhibitors, thus suggesting an important role for nonrenin proteases in vascular angiotensin II formation.

Acknowledgments

We thank Hildeberto Caldo for excellent technical assistance and Dr. Maria A.P. Franco for helpful advice about statistical analysis.
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doi: 10.1161/01.HYP.19.6.575

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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