Vasopeptidase inhibition and Ang-(1-7) in the spontaneously hypertensive rat

CARLOS M. FERRARIO, DAVID B. AVERILL, K. BRIDGET BROSNIHAN, MARK C. CHAPPELL, SAMY S. ISKANDAR, RICHARD H. DEAN, and DEBRA I. DIZ

The Hypertension and Vascular Disease Center, Wake Forest University School of Medicine, Winston-Salem, NC 27157

Vasopeptidase inhibition and Ang-(1-7) in the spontaneously hypertensive rat.

Background. Omapatrilat, a new vasopeptidase inhibitor, inhibits the activity of angiotensin-converting enzyme (ACE) and neutral endopeptidase 24.11 (NEP). Because these two enzymes participate in the degradation of the vasodilator and natriuretic peptide, angiotensin-(1-7) [Ang-(1-7)], we assessed whether omapatrilat treatment is associated with changes in the plasma and urinary excretion rates of the angiotensins.

Methods. We investigated in spontaneously hypertensive rats (SHR) (0.24 kg body weight) the effect of omapatrilat on plasma and urinary concentrations of angiotensin (Ang) I, Ang II and Ang-(1-7) during 17 days of administration of either the drug (N = 15, 100 μmol/kg/day) or vehicle (N = 14) in the drinking water. Hemodynamic and renal excretory function studies were associated with histological examination of the expression of Ang-(1-7) in the kidneys of both vehicle and omapatrilat-treated SHRs.

Results. Omapatrilat induced a sustained lowering of systolic blood pressure (−68 mm Hg) without changes in cardiac rate. The mild positive water balance produced by omapatrilat did not cause natriuresis or kaliuresis, although it was associated with a significant decrease in urine osmolality. Blood pressure normalization was accompanied by increases in plasma Ang I (2969%), Ang II (57%), and Ang-(1-7) (163%) levels, paralleling pronounced increases in urinary excretion rates of Ang I and Ang-(1-7) but not Ang II. Detection of Ang-(1-7) immunostaining in the kidneys of five other SHR exposed either to vehicle (N = 3) or omapatrilat (N = 2) ascertained the source of the Ang-(1-7) found in the urine. Intense Ang-(1-7) staining, more pronounced in omapatrilat-treated SHR, was found in renal proximal tubules throughout the outer and inner regions of the renal cortex and the thick ascending loop of Henle, whereas no Ang-(1-7)-positive immunostaining was found in glomeruli and distal tubules.

Conclusions. Omapatrilat antihypertensive effects caused significant activation of the renin-angiotensin system associated with increases in urinary excretion rates of Ang I and Ang-(1-7). Combined studies of Ang-(1-7) metabolism in urine and immunohistochemical studies in the kidney revealed the existence of an intrarenal source, which may account for the pronounced increase in the excretion rate of the vasodilator heptapeptide. These findings provide further evidence for a contribution of Ang-(1-7) to the regulation of renal function and blood pressure.

Combined inhibition of both angiotensin-converting enzyme (ACE) and neutral endopeptidase 24.11 has been explored as a strategy to achieve a greater antihypertensive effect through inhibition of angiotensin II (Ang II) production and metabolism of atrial natriuretic peptide (ANP) [1, 2]. Omapatrilat is a newly developed vasopeptidase inhibitor with a dual action on both ACE and neutral endopeptidase 24.11 [2]. The potent antihypertensive effects of omapatrilat appear to be greater than those associated with ACE therapy [3–5], a finding that suggests that the mode of action of this drug results from the additional potentiating effect of augmenting the counterbalancing role of the natriuretic peptide system on blood pressure. To the extent that neutral endopeptidase inhibition may also affect the degradation of angiotensin-(1-7) [Ang-(1-7)] [6, 7], we investigated the effects of omapatrilat on the fate of both the plasma and kidney renin-angiotensin systems. The study was prompted by the demonstration that urinary excretion rates of Ang-(1-7) were markedly elevated in salt-sensitive hypertensive subjects in response to oral administration of omapatrilat [1].

METHODS

Experiments were done in 29 aged-matched (10-week-old) male spontaneously hypertensive rats (SHR) (Charles River, Wilmington, MA, USA) in accordance with the guidelines set forth by the Animal Care and Use Committee of the Wake Forest University School of Medicine. At the initiation of the study the body weight of SHR averaged 242 ± 3 g. Rats were housed in metabolic cages (Harvard Bioscience, South Natick, MA, USA) in
a room maintained at 25 ± 2°C on a 12-hour light/dark cycle and fed a powdered rat chow (Purina Mills, Inc., Richmond, IN, USA), providing a daily intake of 17 mEq sodium and 28 mEq potassium per 100 g of solid weight.

Experimental protocol

Rats were assigned randomly to drink either tap water or water to which a solution of omapatrilat was added to provide a daily intake of 100 μmol/kg/day of omapatrilat for up to 17 days.

Twenty-four-hour water and food intake, urine volume, and urine sodium and potassium excretion were measured for 3 days before and at regular intervals after initiation of the drug treatment. Systolic blood pressure was measured before and throughout the treatment period by the tail cuff method (Narco Bio-systems, Houston, TX, USA). Heart rate was derived from counting of the pulse waves visualized in the oscilloscope’s screen. At the completion of the treatment period, rats were sacrificed by decapitation and the heart was removed, blotted dry and weighed.

Laboratory procedures

The concentrations of electrolytes in aliquots of urine were determined using the Nova Biomedical Electrolyte Analyzer (Waltham, MA, USA), whereas the concentration of creatinine was measured photometrically (Sigma Chemical Co., St Louis, MO, USA).

Urinary concentrations of Ang I, Ang II and Ang-(1-7) were determined by radioimmunoassay, as described in detail elsewhere [8]. Urine was extracted using Sep-Pak columns activated with 5 mL sequential washes of a mixture of ethanol:water:4% acetic acid (83:13:4), methanol, ultra-pure water, and 4% acetic acid. The sample was applied to the column, washed with ultra-pure water and acetone and eluted with two 1 mL and one 1.5 mL washes of a mixture of ethanol:water:4% acetic acid. The weight of the eluate was recorded and, from the total eluate, aliquots were transferred into conical bottom polystyrene tubes and dried. The eluted sample was reconstituted into a Tris buffer with 0.1% bovine serum albumin (BSA). The concentrations of angiotensin peptides were measured by radioimmunoassay, using antibodies described previously [9]. Recoveries of radiolabeled angiotensin added to the sample and followed through the extraction averaged 88%. Samples were corrected for recoveries, as described elsewhere [8].

Urine samples collected overnight from untreated SHR rats (10 weeks of age) were pooled and dialyzed against 10 mmol/L N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 125 mmol/L sodium chloride (NaCl) pH 7.4 and concentrated on a 30,000 D filter (Millipore, Bedford, MA, USA). Ang-(1-7) (Bachem, Torrance, CA, USA) was added to 0.5 mL urine (final concentration of 100 μmol/L) in the presence or absence of 1 μmol/L omapatrilat for up to 60 minutes at 37°C. Aliquots were taken at various times into 0.2% phosphoric acid and the metabolism of Ang-(1-7) was determined by high pressure liquid chromatography (HPLC) analysis as described previously [6]. The data were expressed as the percentage of Ang-(1-7) remaining at a given time as compared to control [urine + 0.2% phosphoric acid + Ang-(1-7)]. The half-life (t1/2) of the peptide was calculated from a nonlinear regression exponential decay curve using the Prism plotting and statistical program (Graph Pad, San Diego, CA, USA).

Angiotensin-(1-7) immunocytochemistry

Kidneys from three vehicle-treated and two omapatrilat-dosed animals were placed immediately in a solution of 4% formalin acetate. Tissue was left in 4% formalin for 24 to 48 hours before being transferred to 70% ethanol. Kidney tissue was then imbedded in paraffin and five μm sections were transferred to subbed slides deparaffinized by sequential washes with xylene, 100% ethanol, 95% ethanol, 75% ethanol, and double distilled water. Tissue sections were then incubated with 3% hydrogen peroxide for 5 minutes, washed with phosphate-buffered saline (PBS; pH 7.2), dried, and incubated with 5% formalin goat serum for 1 hour at room temperature. Sections were then washed with PBS and incubated overnight at 4°C with an affinity-purified rabbit polyclonal antibody to Ang-(1-7) at 1:25 dilution of the antibody in 1% BSA. The Ang-(1-7) antibody was purified and characterized, as described elsewhere [10]. The next day, tissues were washed with PBS and for 3 hours at 4°C with a biotinylated anti-rabbit antibody at a dilution of 1:400 in 1% BSA. Slides were rinsed with PBS, blotted dry, and reacted immunocytochemically using the avidin-biotin method (Vector Laboratories, Burlingame, CA, USA) [11] and stained brown with 3’3’-diaminobenzidine (Sigma Chemical Co.) in Tris-buffered saline (0.05 mol/L, pH 7.6-7.7). The reaction was stopped in PBS and sections rinsed in double distilled water before being counterstained with Harris hematoxylin (Sigma Chemical Co.). Tissue sections were dehydrated in ethanol (70% to 100%) and then rinsed in Histoclear (National Diagnostics, Atlanta, GA, USA). Finally, they were cover-slipped using Histomount (National Diagnostics). The specificity of the staining using the Ang-(1-7) antibody was determined by pre absorption of the Ang-(1-7) antibody with 10 μmol/L Ang-(1-7). Nonimmune rabbit serum was further used as a control for the validity of the staining procedure.

Statistical analysis

All data are expressed as mean ± SE. Differences across time for both the treatment and control groups analyzed by repeated measures ANOVA followed by
Table 1. Baseline values in spontaneously hypertensive rats given vehicle or omapatrilat

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vehicle (N = 14)</th>
<th>Omapatrilat (N = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure mm Hg</td>
<td>184 ± 2</td>
<td>192 ± 3</td>
</tr>
<tr>
<td>Heart rate beats/min</td>
<td>264 ± 6</td>
<td>269 ± 7</td>
</tr>
<tr>
<td>Water intake mL</td>
<td>26.3 ± 1.58</td>
<td>27.4 ± 1.42</td>
</tr>
<tr>
<td>Urinary output mL</td>
<td>9.91 ± 1.06</td>
<td>10.30 ± 1.06</td>
</tr>
<tr>
<td>Creatinine excretion mg/dL</td>
<td>115 ± 32</td>
<td>120 ± 15</td>
</tr>
<tr>
<td>Urinary Ang I excretion fmol/24 hours</td>
<td>3932 ± 359</td>
<td>4509 ± 635</td>
</tr>
<tr>
<td>Urinary Ang II excretion fmol/24 hours</td>
<td>840 ± 140</td>
<td>1043 ± 130</td>
</tr>
<tr>
<td>Urinary Ang-(1-7) excretion fmol/24 hours</td>
<td>3755 ± 471</td>
<td>4260 ± 422</td>
</tr>
<tr>
<td>Urinary sodium excretion mEq/24 hours</td>
<td>1.69 ± 0.09</td>
<td>1.77 ± 0.08</td>
</tr>
<tr>
<td>Urinary potassium excretion mg/dL</td>
<td>2.44 ± 0.30</td>
<td>2.90 ± 0.16</td>
</tr>
<tr>
<td>Urinary osmolality mOsm/L</td>
<td>1841 ± 80</td>
<td>1811 ± 104</td>
</tr>
<tr>
<td>Body weight g</td>
<td>242 ± 4</td>
<td>242 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

Scheffes post hoc test. Differences between groups were analyzed by the Student t test and P values less than or equal to 0.05 were considered statistically significant.

RESULTS

Hemodynamic and renal excretory effects of omapatrilat

Baseline hemodynamic, water intake, and urinary electrolyte and excretion variables did not differ between SHR randomized to receive either tap water or omapatrilat (Table 1). As illustrated in Figure 1, SHR medicated with omapatrilat showed a progressive and significant decrease in systolic blood pressure that reached a plateau within 10 days after commencement of the treatment period. On day 16, average systolic blood pressure in omapatrilat-treated rats was 122 ± 5 mm Hg compared to 190 ± 3 mm Hg in vehicle-treated rats (P < 0.001). The antihypertensive response was associated with no changes in heart rate (Fig. 1), but a significant decrease in heart weight (812 ± 27 mg compared to 977 ± 31 mg in vehicle-treated controls, P < 0.02). There were no differences in body weights of rats randomized to either vehicle (273 ± 5 g) or omapatrilat (265 ± 5 g, P > 0.05) at the end of the treatment regimen and the medication had no significant effect on food intake when compared to vehicle-treated rats.

Plasma levels of angiotensin peptides in vehicle- and omapatrilat-treated rats at the completion of the treatment period are illustrated in Figure 2. Omapatrilat caused large elevations in plasma concentrations of Ang I, Ang II, and Ang-(1-7), amounting to 2969%, 57%, and 163% of values found in vehicle-treated SHR, respectively. The rise in Ang I concentrations resulted in a significant decrease in the Ang II/Ang I ratio to values averaging 0.15 ± 0.04 in omapatrilat-treated rats compared to an Ang II/Ang I ratio of 1.73 ± 0.39 in vehicle-treated controls (P < 0.001). In addition, omapatrilat caused a significant increase in plasma concentrations of angiotensins [Ang I, Ang II, and Ang-(1-7)] at day 17 of treatment period. Values are means ± SE of SHR given either vehicle (■) or omapatrilat (●). *P < 0.01 compared to vehicle.
trilat reduced the Ang-(1-7)/Ang I in omapatrilat-treated rats averaged 0.11 ± 0.02 compared to 1.09 ± 0.17 in vehicle-treated controls (P < 0.001).

Omapatrilat treatment resulted in a progressive rise in cumulative fluid balance as water intake was significantly augmented over the accompanying increases in urinary volume (Fig. 3). In omapatrilat-treated rats, water intake increased from 27.45 ± 1.42 mL/24 hours to 49.87 ± 2.19 mL/24 hours (P < 0.002), whereas it did not change in SHR given the vehicle (26.30 ± 1.58 mL/24 hours compared to 25.95 ± 0.98 mL/24 hours, P > 0.05) at the end of the study period. Figure 3 also shows that the polyuria in omapatrilat-treated SHR was associated with a significant and sustained fall in urinary osmolality, whereas urinary excretion rates of either sodium or potassium did not change.
At baseline, angiotensin peptides were detected in the urine of both vehicle and omapatrilat-treated SHR at concentrations not different from those previously reported elsewhere [12]. Initiation of omapatrilat treatment was associated with significant increases in the excretion rates of Ang I and Ang-(1-7), whether the data were expressed either in terms of concentrations or in terms of 24-hour excretion rates (Fig. 4). Changes in the excretion rate of urinary Ang II were not statistically significant ($P > 0.05$).

Multiple regression analysis of the combined data from vehicle- and omapatrilat-treated SHR (Table 2) showed that systolic blood pressure was negatively correlated with heart rate, urinary volume, urine osmolality, water intake, and urinary excretion rates of Ang I and Ang-(1-7). Systolic blood pressure showed a direct correlation with urinary osmolality, an inverse correlation with both urine volume and Ang-(1-7), and no significant correlation with Ang II.

**Evaluation of the source of Ang-(1-7) in urine**

The rate of Ang-(1-7) hydrolysis in the urine of untreated SHR in the absence and presence of 1 μmol/L omapatrilat was evaluated in dialyzed urine collected from untreated SHR. As shown in the chromatograph (Fig. 5A), Ang-(1-7) added to urine was hydrolyzed primarily to Ang-(1-4) and Ang-(1-5). Addition of omapatrilat essentially abolished production of both metabolites, while substantially increasing the peak of Ang-(1-7) in the chromatogram. The time course for the metabolism of Ang-(1-7) in urine before (control) or after addition of omapatrilat is documented (Fig. 5B). Incubation of urine with omapatrilat caused a six-fold increase (5.2 minutes vs. 33 minutes) in the Ang-(1-7) half-life.

A composite of sections of kidneys obtained from SHR is shown given either vehicle (Fig. 6A and C) or omapatrilat (Fig. 6B and D) in their drinking water for 17 days. Ang-(1-7) immunostaining was found throughout the renal cortex but absent in renal glomeruli. At higher magnification, Ang-(1-7) immunostaining was visualized in the cytoplasm of renal proximal convoluted tubules with a tendency for increased positive staining around the basolateral and brush border of the proximal tubules (Fig. 6C). No positive immunostaining is found in distal tubules. Figure 6D shows Ang-(1-7) labeling of thick ascending limbs of loops of Henle in omapatrilat-treated SHR. Pre-absorption of the Ang-(1-7) antibody with synthetic Ang-(1-7) and nonimmune rabbit serum yielded no staining in all sections examined (Fig. 7).

**DISCUSSION**

These studies both confirm [4] and extend knowledge of the effects of vasopeptidase inhibition in rats with spontaneous hypertension by demonstrating (1) a strong association of the antihypertensive effect of the drug with increases in the plasma concentrations of the angiotensins and increases in urinary excretion rates of Ang I and Ang-(1-7); and (2) evidence that the changes in urinary Ang-(1-7) reflect an intrarenal mechanism of Ang-(1-7) secretion by renal tubules. Hemodynamically,
Table 2. Multiple regression analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Heart rate</th>
<th>Urine volume</th>
<th>Urine osmolality</th>
<th>Water intake</th>
<th>Urinary Ang I</th>
<th>Urinary Ang II</th>
<th>Urinary Ang-(1-7)</th>
<th>Systolic pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate beats/min</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine volume mL</td>
<td>0.51</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine osmolality mOsm/L</td>
<td>-0.65</td>
<td>-0.94</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water intake mL</td>
<td>0.55</td>
<td>0.99</td>
<td>-0.93</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary Ang I fmol/mg</td>
<td>0.35</td>
<td>0.76</td>
<td>-0.77</td>
<td></td>
<td>0.77</td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>Urinary Ang II fmol/mg</td>
<td>0.18</td>
<td>0.55</td>
<td>-0.49</td>
<td></td>
<td>0.54</td>
<td>0.71</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Urinary Ang-(1-7) fmol/mg</td>
<td>0.49</td>
<td>0.98</td>
<td>-0.93</td>
<td></td>
<td>0.96</td>
<td>0.81</td>
<td>0.54</td>
<td>1.00</td>
</tr>
<tr>
<td>Systolic pressure mm Hg</td>
<td>-0.51</td>
<td>-0.97</td>
<td>0.93</td>
<td>-0.98</td>
<td>-0.80</td>
<td>-0.48</td>
<td>-0.94</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Multiple regression analysis of combined data from vehicle- and omapatrilat-treated rats. Numbers in bold are statistically significant at a P value of at least 0.05.

Fig. 5. Comparison of absorbance using high pressure liquid chromatography.

(A) High pressure liquid chromatography (HPLC) of angiotensin-(1-7) [Ang-(1-7)] metabolism in dialyzed rat urine for 15 minutes at 37°C reveals conversion to Ang-(1-4) and Ang-(1-5) [control, solid lines]. Addition of one μmol/L omapatrilat [OMAP, dashed lines] attenuated generation of these angiotensin metabolites and increased the recovery of Ang-(1-7). (B) Time course for the hydrolysis of Ang-(1-7) at 37°C in rat urine alone (○) or in the presence of omapatrilat (●).

The sustained antihypertensive effect of omapatrilat in SHR was associated with increases in plasma Ang I, Ang II, and Ang-(1-7) levels, a finding that may reflect a combination of enhanced renin release and activation of a non-ACE-dependent pathway for Ang II formation following inhibition of ACE [20, 21]. The increase in plasma Ang-(1-7) levels is in keeping with the finding that ACE is the enzyme responsible for the degradation of the peptide into Ang-(1-5) [22], and that both vascular endothelium prolyl endopeptidase (E.C. 3.4.24.26) and smooth muscle thimet oligo peptidase (E.C. 3.4.24.15) are capable of forming Ang-(1-7) [23, 24]. Therefore, the effect of omapatrilat on inhibiting both ACE and neutral endopeptidase 24.11 blocks the primary pathway for the degradation of Ang-(1-7), while allowing alternative Ang-(1-7)-forming enzymes to act on the increased levels of Ang I substrate. This interpretation agrees also with the finding that inhibition of ACE is associated with increased prolyl endopeptidase-like activity in plasma and lung [25].

We have previously documented that endothelial-derived ACE cleaves the Ile5 - His6 bond of Ang-(1-7) to render Ang-(1-5), whereas in the kidney, neutral endopeptidase 24.11 generates Ang-(1-4) through cleavage of the Tyr4 – Ile5 bond of Ang-(1-7) [6, 7, 22]. The metabo-
Fig. 6. Photomicrographs of sections of kidneys. SHR given either vehicle (A) and (C) or omapatrilat (B) and (D) for 17 days. (A) and (C) from a vehicle-treated SHR shows that Ang-(1-7) immunostaining is present in renal proximal convoluted tubules throughout the cortex while the medulla showed scant Ang-(1-7)-positive staining cells. No immunopositive Ang-(1-7) staining is found in glomeruli. (B) shows an apparent increase in the density of Ang-(1-7) immunostaining in renal proximal tubules, including increased immunostaining now also found in the thick portion of the ascending limb of Henle’s loops (D). Magnification for (A) and (B) ×1100; magnification for (C) and (D) ×100.

The observation that the profuse diuresis produced by
omapatrilat was accompanied by a significant decrease in urine osmolality, but maintenance of water and electrolyte balance is a new observation that implicates suppression of vasopressin as a component of the endocrine response resulting from blockade of neutral endopeptidase 24.11. In previous studies, we showed that the transient diuresis and natriuresis produced for the first 3 days of a 14-day continuous intravenous infusion of Ang-(1-7) in SHR was accompanied by suppression of plasma vasopressin concentrations and higher urinary excretion rates of 6-keto prostaglandin F1α (6-keto-PGF1α) [31]. These data suggest that local production of prostaglandins may also contribute to inhibition of vasopressin action on renal tubules, an interpretation that agrees with the previous demonstration that Ang-(1-7) has a potent stimulatory effect on release of arachidonic acid in isolated renal tubules [32]. This possibility is also in keeping with the demonstration of Ang-(1-7)-positive immunoreactive material in the ascending limb of Henle. The persistence of diuresis but not natriuresis in SHR given omapatrilat suggests an intervening contribution of both bradykinin and atrial natriuretic factor acting to offset the normal control mechanisms that would allow escape from the diuresis, especially in the face of decreased pressure. In keeping with this interpretation, the diuretic effect of kinins is more pronounced than its natriuretic effects [33].

CONCLUSION

In summary, the strong correlation between the antihypertensive response to omapatrilat and the increases in urinary excretion rates of Ang I and Ang-(1-7) would suggest a contribution of Ang-(1-7) to the vasodilator response mediated by this agent. Ang-(1-7) has been shown to act as a systemic vasodilator [34] and stimulate diuresis and natriuresis through inhibition of tubular sodium/potassium ATPase pump and the sodium/hydrogen exchanger [14]. While urinary Ang-(1-7) levels are markedly reduced in untreated essential hypertensive subjects, chronic administration of ACE inhibitors is associated with significant rises in urinary Ang-(1-7) [30]. The important association between increases in both plasma and urinary Ang-(1-7) and the antihypertensive effect of omapatrilat suggests, but obviously does not prove, that this humoral regulator may play a contributing role in the mechanisms that account for the control of blood pressure. In support for this hypothesis, administration of omapatrilat to salt-sensitive hypertensive subjects is associated with sustained increases in urinary excretion rates of both Ang I and Ang-(1-7) [4]. In fact, our study provides another example where significant correlations were found between the increases in urinary Ang-(1-7) and normalization of blood pressure, whereas no significant correlations can be made for Ang II. While there is no reason to suspect that the inhibitory effect of omapatrilat on the metabolism of the atrial natriuretic peptide is not a mechanism contributing to the antihypertensive effect of the agent, our data now implicate Ang-(1-7) as an additional factor contributing to the potent antihypertensive effects of omapatrilat. The detection of Ang-(1-7) in renal tubules and collecting ducts of SHR is a new and important finding providing an underlying clue for the further and more precise investigation of the effects of Ang-(1-7) in the regulation of renal function and tubular mechanisms controlling the excretion of solutes and electrolytes.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of Ms. Shakeela Pitts in the conduction of these experiments. This work was supported in part by NIH grants HL-51952, HL-68258, and AHA-151521 from the American Heart Association and an unrestricted grant from the Bristol Myers Research Institute (Providence, NJ). Powder omapatrilat was a generous gift of Bristol Myers Squibb Corporation (Providence, NJ).

Reprint requests to Carlos M. Ferrario, M.D., Hypertension and Vascular Disease Center, Wake Forest University School of Medicine, Winston-Salem, NC 27157.

Email: cferrari@wfubmc.edu

REFERENCES

14. Handa RK, Ferrario CM, Strandhov JW: Renal actions of angio-