Effects of a Metalloendopeptidase-24.15 Inhibitor on Renal Hemodynamics and Function in Rats

Xiao-Ping Yang, Shigeyuki Saitoh, A. Guillermo Scicli, Edward Mascha, Marian Orlowski, Oscar A. Carretero

Abstract

N-[1-(S,S)-carboxyl-3-phenylpropyl]- Ala-Ala-Phe-p-aminobenzoate (cFP-AAF-pAB), an active-site-directed inhibitor of metalloendopeptidase-24.15, has been shown to lower blood pressure, increase cardiac output and renal blood flow, and potentiate the intravenous bradykinin-induced vasodepressor response. Because in vivo cFP-AAF-pAB can be converted to N-[1-(S,S)-carboxyl-3-phenylpropyl]-Ala-Ala (a compound with angiotensin converting enzyme inhibitory activity) by metalloendopeptidase-24.11, it is possible that some of its effects are due to angiotensin converting enzyme inhibition. In the present study, we questioned (1) whether cFP-AAF-pAB inhibits angiotensin converting enzyme in vivo and (2) whether cFP-AAF-pAB has renal effects that are independent of its conversion to an angiotensin converting enzyme inhibitor. cFP-AAF-pAB alone (3 μmol in 300 μL per rat) almost abolished the blood pressure response to angiotensin I, suggesting that in vivo it inhibits angiotensin converting enzyme. In rats pretreated with a high dose of enalaprilat (1 mg/kg), cFP-AAF-pAB had no further effect on blood pressure, renal blood flow, or potentiation of the vasodepressor response to bradykinin but still increased glomerular filtration rate by 44±9% (P<0.01); urine volume increased by 118±10% (P<0.001), urinary sodium excretion by 230±31% (P<0.001), urinary potassium excretion by 68±14% (P<0.01), and urinary cyclic GMP by 55±18% (P<0.01). All of these changes were significant compared with enalaprilat/vehicle-treated rats. Fractional excretion of sodium and potassium did not differ from controls. These results suggest that effects of cFP-AAF-pAB on blood pressure, renal blood flow, and potentiation of the vasodepressor response to bradykinin could be mediated by angiotensin converting enzyme inhibition. However, some of the renal effects may be due to inhibition of metalloendopeptidase-24.15 or peptides other than angiotensin converting enzyme. (Hypertension. 1994;23[suppl I]:I-235-I-239.)

Key Words • membrane metalloendopeptidase • protease inhibitors • dipeptidyl peptidases • angiotensin converting enzyme inhibitors • kininase II • enalaprilat • renal circulation • hemodynamics

Metalloendopeptidase-24.15 (MEP-24.15) is a Zn\(^{2+}\)-containing neutral endopeptidase that is highly concentrated in the brain and testes but is also found in the kidney, heart, lung, and liver.\(^{1-3}\) In vitro, it degrades a variety of peptides such as bradykinin, angiotensin I (Ang I), neurotensin, and luteinizing hormone–releasing hormone (LHRH) and converts enkephalin-containing peptides into Leu- and Met-enkephalin.\(^{2,4}\) In vivo, N-[1-(S,S)-carboxyl-3-phenylpropyl]-Ala-Ala-Phe-p-aminobenzoate (cFP-AAF-pAB) blocks degradations of LHRH (a substrate for MEP-24.15); however, its physiological role in the metabolism of vasoactive peptides has not been determined. Genden and Molinieux\(^{5}\) recently reported that inhibition of MEP-24.15 by cFP-AAF-pAB, an active-site–directed inhibitor, produced a marked fall in mean blood pressure (MBP) that was almost abolished by a kinin receptor antagonist. cFP-AAF-pAB also potentiated the bradykinin-induced vasodepressor response. In preliminary studies,\(^{6,7}\) we found that cFP-AAF-pAB lowers MBP and increases cardiac output, renal blood flow (RBF), and plasma kallikrein. A recent report\(^{8}\) states that in vitro cFP-AAF-pAB competitively inhibits purified angiotensin converting enzyme (ACE) with a K\(_i\) of 0.2 μmol/L and also inhibits serum ACE activity. This could be due to conversion of cFP-AAF-pAB to N-[1-(S,S)-carboxyl-3-phenylpropyl]-Ala-Ala (cFP-AA), a compound having ACE inhibitory activity, by MEP-24.11\(^2\) (enkephalinase) (Cardozo and Orlowski, unpublished observations). Thus, although cFP-AAF-pAB has no intrinsic ACE inhibitory activity, conversion to cFP-AA might be primarily responsible for the effects of cFP-AAF-pAB in vivo.

To see whether cFP-AAF-pAB inhibits ACE in vivo, we studied its effect on the MBP response to Ang I. To see whether cFP-AAF-pAB has effects independent of ACE inhibition, we studied its effects on MBP, the MBP response to bradykinin, and its renal effect in rats after blocking ACE with a high dose of enalaprilat.

Methods

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass) weighing 285 to 300 g were maintained in an air-conditioned room and given standard rat chow (0.4% NaCl) and free access to tap water.

cFP-AAF-pAB was synthesized by M. Orlowski et al. Ang I and angiotensin II (Ang II) were purchased from Peninsula Laboratories, Belmont, Calif; bradykinin was purchased from Bachem, Torrance, Calif; and enalaprilat was provided by Merck Sharp & Dohme Research Laboratories, Rahway, NJ.
[\textsuperscript{3}H]inulin and [\textsuperscript{14}C]para-aminomhippuric acid (PAH) were purchased from Du Pont, Boston, Mass.

All surgical preparations and experiments were performed with rats under sodium pentobarbital anesthesia (50 mg/kg). Seven days before the experiment, a stainless steel catheter (16-gauge needle with a silicone elastomer [Silastic] sleeve and the proximal end covered by a cylindrical Silastic dome) was implanted into the bladder via a midline incision. On the day of the experiment, two polyethylene tubes (PE-10, Clay Adams, Parsippany, NJ) were inserted into the abdominal aorta and inferior vena cava via the femoral artery and vein for (1) measurement of MBP and (2) blood withdrawal, drug administration, and saline infusion, respectively.

MBP was measured and recorded on a recorder (Gould Brush 220, Gould Instruments, Cleveland, Ohio). Urine samples were collected via the bladder catheter, and urine volume (UV) was determined gravimetrically. Urinary and plasma [\textsuperscript{3}H]inulin and [\textsuperscript{14}C]PAH were measured with a liquid scintillation counter (Beckman Instruments, Irvine, Calif). Glomerular filtration rate (GFR) and renal plasma flow (RPF) were calculated by the ratio of urine to plasma inulin or PAH concentration, respectively, and multiplied by UV. RBF was calculated from the formula $RBF = \frac{RPF}{(1 - \text{Hematocrit})}$. Fractional excretion of sodium ($\text{FENa}$) and potassium ($\text{K^+}$) concentrations were measured with a NOVA 1 ion-electrode analyzer (NOVA Biochemical, Newton, Mont). Urinary and plasma osmolarity were measured with a freezing-point osmometer. Plasma atrial natriuretic factor (ANF) and urinary cyclic GMP ($\text{cGMP}$) excretion were measured by radioimmunoassay. This study was approved by the Henry Ford Hospital Care of Experimental Animals Committee.

**Protocol 1: Effect of cFP-AAF-pAB on Mean Blood Pressure Response to Intravenous Ang I, Ang II, and Bradykinin**

To test whether cFP-AAF-pAB blocks the Ang I-induced vaspressor response, we obtained dose-response curves for Ang I (25, 50, and 100 ng/100 \mu L per rat) before and after administration of cFP-AAF-pAB (3 \mu mol per rat in 300 \mu L saline). Injections of Ang I were spaced 10 minutes apart in random succession (n=6). The effect of cFP-AAF-pAB on the MBP response to bradykinin (100 ng per rat) and Ang II (100 ng per rat) was tested in another group of rats (n=6).

**Protocol 2: Effect of cFP-AAF-pAB on Mean Blood Pressure and Renal Hemodynamics and Function in Non-Pretreated Rats**

Rats were divided into two groups: (1) vehicle (300 \mu L saline, n=10) and (2) cFP-AAF-pAB (n=14). The experiment consisted of a 1-hour equilibration period followed by 30-minute control and experimental periods. Saline containing [\textsuperscript{3}H]inulin and [\textsuperscript{14}C]PAH (2.5 \mu Ci/mL) was infused from the proximal end of each catheter. The experiment consisted of a 1-hour equilibration period followed by 30-minute control and experimental periods. Saline containing [\textsuperscript{3}H]inulin and [\textsuperscript{14}C]PAH (2.5 \mu Ci/mL) was infused from the proximal end of each catheter. Injections of Ang I were spaced 10 minutes apart in random succession (n=6). The effect of cFP-AAF-pAB on the MBP response to bradykinin (100 ng per rat) and Ang II (100 ng per rat) was tested in another group of rats (n=6).

**Protocol 3: Effect of cFP-AAF-pAB on Mean Blood Pressure and Renal Hemodynamics and Function in Rats Pretreated With Enalaprilat**

This protocol was similar to protocol 1 except that rats were pretreated with enalaprilat immediately before the equilibration period (1 mg/kg given as a bolus). Rats were divided into two

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**Results**

**Protocol 1**

Intravenous Ang I produced a dose-dependent pressure response, which was almost abolished by cFP-AAF-pAB (average slope: 3.2±0.8 versus 11.0±2.4; P<.01) (Fig 1, top). cFP-AAF-pAB also potentiated the intravenous bradykinin-induced vasodepressor response (−1±1 versus −31±4 mm Hg; P<.001) (Fig 1, bottom) but did not modify the MBP response to Ang II (67±2.4 versus 63±1.4, P=NS).

**Protocol 2**

The Table shows that in non-pretreated rats, cFP-AAF-pAB slightly but significantly lowered MBP and increased
GFR, RBF, UV, and UN,V. All of these changes were significant compared with vehicle treatment. There was no significant change in UK, FEN,, or FER. Urinary osmolarity was significantly decreased by cFP-AAF-pAB.

**Protocol 3**

Enalaprilat itself decreased MBP by 15 to 20 mm Hg in both groups; it returned to baseline within 20 to 30 minutes. cFP-AAF-pAB had no further effect on MBP and RBF (Fig 2); however, GFR and UV were significantly increased by 44±9% and 118±10.4% (P<.01 and <.001, respectively), whereas there was no change in the vehicle-treated rats. UN,V and U,K were significantly increased in both groups; however, the increase was much greater in cFP-AAF-pAB--than in vehicle-treated rats (by 230±31% versus 107±15% and 68±14% versus 26±11%; P<.001 and <.01, respectively). FEK was increased significantly (but to the same degree) in both groups. FEK was increased in both cFP-AAF-pAB--and vehicle-treated rats, although the changes were not statistically significant. cFP-AAF-pAB increased urinary osmolarity by 17±4% (P<.05) and increased urinary cGMP excretion from 23±1.7 to 35±4.2 pmol/mL per minute per kilogram (P<.01). There was no change in plasma osmolarity, Na⁺, K⁺, or ANF. At the end of the experiment, the MBP response to Ang I was blocked in both groups (Fig 3, bottom), indicating inhibition of ACE throughout. There were dose-dependent vasodepressor responses to bradykinin and vasopressor responses to Ang II (Fig 3); however, they did not differ between groups.

**Discussion**

The present study suggests that cFP-AAF-pAB, an active-site-directed MEP-24.15 inhibitor, has renal effects that are not mediated by ACE inactivation, because enalaprilat did not diminish its influence on GFR, UV, UN,V, and U,K. Thus, MEP-24.15 may play a role in the regulation of renal function by catabolizing vasoactive peptides in the kidney.

MEP-24.15 was first identified by Orlowski et al in 1983 as a neutral endopeptidase; it is highly concentrated in the brain and testes but is also found in the kidney, heart, lung, and liver as a soluble enzyme.1-3 In vitro MEP-24.15 degrades several peptides, including bradykinin, angiotensin, neurotensin, and LHRH, and converts proenkephalins into bioactive enkephalins.2-4 Its role in the metabolism of vasoactive peptides in vivo is still not clear. Recently, Genden and Molineaux reported that cFP-AAF-pAB lowered MBP in normotensive rats and that this vasodepressor effect was almost abolished by a kinin receptor antagonist. In addition, cFP-AAF-pAB potentiated the vasodepressor response to bradykinin. We extended these studies and found that cFP-AAF-pAB decreases MBP, increases cardiac output and RBF, and tends to increase plasma kinins, resulting in a significant decrease in renal and total vascular resistance.5,7 Taken together, these data suggest that MEP-24.15 is a peptidase implicated in the metabolism of vasoactive peptides in vivo, and as such contributes to the control of circulatory hemostasis. However, Chappell et al recently reported that in vitro cFP-AAF-pAB inhibits purified ACE with a Kᵢ of 0.2 μmol/L and also competitively inhibits serum ACE activity. Cardozo and Orlowski (unpublished observation) found that in vitro cFP-AAF-pAB at concentrations of up to 10 μmol/L failed to inhibit ACE if an inhibitor of MEP-24.15 was added to the incubation medium. Therefore, it is possible that the commercial ACE preparation (from Sigma Chemical Co, St Louis, Mo) used by Chappell was contaminated by MEP-24.15, which cleaves cFP-AAF-pAB to cFP-AA, a compound having ACE inhibitory activity. Nevertheless, we found that cFP-AAF-pAB blocked the MBP response to Ang I, suggesting that in vivo it inhibits ACE; this may be due to conversion of cFP-AAF-pAB to cFP-AA by MEP-24.15 or other enzymes or to a direct effect. Regardless of the mechanism, it is important to determine whether the hypotensive and renal effects of cFP-AAF-pAB are solely due to ACE inhibition or in part to its effect on MEP-24.15. Our data indicate that

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**Table 1:** Effects of cFP-AAF-pAB on Renal Hemodynamics and Function in Non-Pretreated Normotensive Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle (n=10)</th>
<th>cFP-AAF-pAB (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>97±4</td>
<td>98±4</td>
</tr>
<tr>
<td>GFR, (mL/min)/kg</td>
<td>9.0±1.6</td>
<td>8.8±1.1</td>
</tr>
<tr>
<td>RBF, (mL/min)/kg</td>
<td>45±7</td>
<td>43±4</td>
</tr>
<tr>
<td>UV, (μL/min)/kg</td>
<td>49±21</td>
<td>63±25</td>
</tr>
<tr>
<td>U₁UV (μmol/L)/min/kg</td>
<td>4.8±2.3</td>
<td>5.8±2.5</td>
</tr>
<tr>
<td>U₂V, (μmol/L)/min/kg</td>
<td>3.9±0.9</td>
<td>4.2±0.7</td>
</tr>
<tr>
<td>FE₅, %</td>
<td>0.4±0.1</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>FE₆, %</td>
<td>18.6±2.6</td>
<td>18.6±2.3</td>
</tr>
<tr>
<td>U₅, mOsm/kg H₂O</td>
<td>1141±155</td>
<td>1357±164</td>
</tr>
</tbody>
</table>

MBP indicates mean blood pressure; GFR, glomerular filtration rate; RBF, renal blood flow; UV, urine volume; U₁UV, urinary sodium excretion; U₂V, urinary potassium excretion; FE₅, fractional excretion of sodium; FE₆, fractional excretion of potassium; and U₅, urinary osmolality.

$P<.05, *P<.01$ vs before cFP-AAF-pAB.

$P<.05, §P<0.1$ vs after vehicle.
the vasodepressor effect of cFP-AAF-pAB, as well as its potentiating effect on the MBP response to bradykinin is mediated by ACE inhibition, because these effects were abolished when ACE was inactivated. However, the renal effects of cFP-AAF-pAB do not seem to be mediated by ACE inhibition but rather are attributable to inhibition of MEP-24.15 or some peptidase other than ACE, because the increase in GFR and excretion of water, Na+, and K+ still persisted when ACE was inactivated by enalaprilat at a dose that resulted in a far higher concentration than its K_i for ACE. It is possible that MEP-24.15 is involved in the metabolism of intrarenal kinins. cFP-AAF-pAB may protect kinins from degradation in the lumen of the distal nephron, thereby affecting water and Na+ excretion.

In the present study, in which rats were saline expanded (100 μL/min for 2 hours), cFP-AAF-pAB had less effect on MBP (−6±1.2 mm Hg) than was found in a previous study (−13±2.2 mm Hg). One explanation could be that volume expansion suppressed plasma renin activity. Because the vasodepressor effect of cFP-AAF-pAB appears to be due to ACE inhibition, this effect will be less potent when the contribution of the renin-angiotensin system to the maintenance of MBP is less. The profound diuretic and natriuretic effects of cFP-AAF-pAB may be attributable to the increase in GFR; however, a tubular action cannot be completely excluded, because there were increases in FE_K and FE_Na, although they did not attain statistical significance. It is also possible that cFP-AAF-pAB acts preferentially in the medulla and redistributes RBF, thereby increasing fractional electrolyte excretion. However, the mechanisms by which cFP-AAF-pAB increases GFR are not clear. One hormone that causes vasodilation, increases GFR, and inhibits Na+ reabsorption is ANF. Like kinins and angiotensin, which are cleaved by a number of peptidases, ANF is also degraded by several endopeptidases. In the kidney, it is hydrolyzed mainly by MEP-24.11 (enkephalinase). We found that cFP-AAF-pAB increases urinary cGMP excretion, which may indicate that MEP-24.15 is involved in the hydrolysis of ANF. However, cFP-AAF-pAB did not
increase plasma ANF concentration. Because cFP-AAF-pAB is a substrate for MEP-24.11, it is unlikely that it inhibits MEP-24.11 at the dose used in the present study. Further studies to demonstrate that cFP-AAF-pAB does not potentiate the renal effect of ANF or that its effects persist in the presence of an ANF antagonist will indicate whether the renal effect of cFP-AAF-pAB is mediated by inhibition of MEP-24.11. As with other metalloendopeptidases (ACE and MEP-24.11), MEP-24.15 is not specific for a particular substrate, such as kinins, but cleaves a number of peptides containing hydrophobic amino acids; therefore, the effects of cFP-AAF-pAB in vivo could be complex.

In summary, the MEP-24.15 inhibitor cFP-AAF-pAB potentiates the vasodepressor response to intravenous bradykinin, decreases MBP, and increases RBF, which may be due to ACE inactivation. However, the increases in GFR, UV, U_nV, and U_kV are independent of ACE inhibition, because they persisted in the presence of an ACE inhibitor. cFP-AAF-pAB might act on MEP-24.15 or peptides other than ACE to inhibit degradation of vasoactive peptides that participate in the regulation of renal function.

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Effects of a metalloendopeptidase-24.15. Inhibitor on renal hemodynamics and function in rats.

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