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-(R,S)-carboxyl-3-phenylpropyl]-Ala-Ala-Phe-β-aminoazobenzate (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-(R,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<.01); urine volume increased by 118±10% (P<.001), urinary sodium excretion by 230±31% (P<.001), urinary potassium excretion by 68±14% (P<.001), and urinary cyclic GMP by 55±18% (P<.001). 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 Zn²⁺-containing neutral endopeptidase that is highly concentrated in the brain and testes but is also found in the kidney, heart, lung, and liver. Recent studies have shown that it degrades a variety of peptides such as bradykinin, angiotensin I (Ang I), and luteinizing hormone-releasing hormone (LHRH) and also inhibits serum ACE activity. This compound having ACE inhibitory activity, by MEP-24.112 (enkephalinase) (Cardozo and Orlowski, unpublished observations). Thus, although cFP-AAF-pAB has no intrinsic ACE inhibitory activity, conversion to cFP-AAA 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.
Intravenous Ang I produced a dose-dependent pressor response, which was almost abolished by cFP-AAF-pAB. The pressor response to Ang II (12.5, 25, 50, and 100 ng per rat) and bradykinin (12.5, 25, 50, and 100 ng per rat) was tested at the end of the experiment. Urinary cGMP excretion and plasma ANF were measured in separate rat groups (cFP-AAF-pAB, n=11; vehicle, n=10). For this purpose, urine was collected in a test tube containing the phosphodiesterase inhibitor IBMX (0.1 mmol/L). Blood samples were withdrawn at the end of the experiment for ANF assay.

**Statistical Analysis**

Results are expressed as mean±SEM. The significance level for each hypothesis is .05; adjustment for multiple testing was made whenever appropriate. Effects of cFP-AAF-pAB were tested by comparing experimental and control groups with either repeated-measures analysis of covariance (adjusting for baseline) or Student’s t test. In protocols testing dose response, the interaction between dose and treatment was tested; if it was significant, appropriate within-group and within-dose analyses were performed.

**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 UNV. All of these changes were significant compared with vehicle treatment. There was no significant change in UK, FEN, or FEK. 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. UNV and USV 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%; P<.01 and <.001, 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 decreased 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, UNV, and USV. 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. In vitro MEP-24.15 degrades several peptides, including bradykinin, angiotensin, neurotensin, and LH-RH, and converts proenkephalins into bioactive enkephalins. 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. 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.
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 Kᵢ 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+ excretion910; however, this is unlikely because there is a great possibility that cFP-AAF-pAB is degraded in the proximal tubule, which is very rich in peptidases. Furthermore, the present results do not support the hypothesis that kinins mediate the effects of cFP-AAF-pAB, because we did not observe a difference in the MBP response to bradykinin between animals treated with enalaprilat alone and combined with cFP-AAF-pAB. Thus, it is unlikely that MEP-24.15 is a major kininase for circulating kinins.

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).7 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 FeNa and FeK, 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.11 Like kinins and angiotensin, which are cleaved by a number of peptidases, ANF is also degraded by several endopeptidase. In the kidney, it is hydrolyzed mainly by MEP-24.11 (enkephalinase).14,15 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-24.11,2 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 (or antibody) or MEP-24.11 inhibitor 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, $U_{Na}V$, and $U_{K}V$ 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 peptidases other than ACE to inhibit degradation of vasoactive peptides that participate in the regulation of renal function.

**Acknowledgment**

This study was supported by grant HL-28982 from the National Institutes of Health, Bethesda, Md.

**References**

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Hypertension, 1994;23:I235
doi: 10.1161/01.HYP.23.1_Suppl.I235

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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