Neutral Endopeptidase Inhibition Augments Vascular Actions of Bradykinin in Patients Treated With Angiotensin-Converting Enzyme Inhibition

Nicholas L.M. Cruden, Keith A.A. Fox, Christopher A. Ludlam, Neil R. Johnston, David E. Newby

Abstract—Angiotensin-converting enzyme and neutral endopeptidase (EC 3.4.24.11; neprilysin) are metalloproteinases present on the endothelium that metabolize bradykinin. Inhibitors of angiotensin-converting enzyme potentiate bradykinin-mediated vasodilation and endothelial tissue plasminogen activator release. Combined angiotensin-converting enzyme and neutral endopeptidase inhibition may have additional beneficial cardiovascular effects mediated through bradykinin potentiation. We investigated the effects of local neutral endopeptidase inhibition on the vascular actions of bradykinin in heart failure patients maintained on chronic angiotensin-converting enzyme inhibition. Ten patients received intrabrachial infusion of thiorphan (30 nmol/min), a neutral endopeptidase inhibitor, in a randomized double-blind placebo-controlled crossover trial. Thiorphan was coinfused with Lys-des-Arg9-bradykinin (1 to 10 nmol/min), bradykinin (30 to 300 pmol/min), atrial natriuretic peptide (10 to 100 pmol/min), and sodium nitroprusside (2 to 8 μg/min). Bradykinin, atrial natriuretic peptide, and sodium nitroprusside caused dose-dependent vasodilatation (peak blood flow 14.4±2.2, 3.6±0.6, and 8.6±1.3 mL per 100 mL/min, respectively; \( P<0.0001 \)). Bradykinin caused dose-dependent increases in tissue plasminogen activator antigen and activity (peak concentration 31.8±3.4 ng/mL and 21.9±7.6 IU/mL, respectively; \( P<0.001 \)) and estimated antigen and activity release (peak release 152±46 ng per 100 mL/min and 154±22 IU/100 mL/min, respectively; \( P<0.005 \)). Compared with placebo, thiorphan augmented bradykinin-mediated vasodilatation (1.4-fold; \( P<0.0001 \)) and net tissue plasminogen activator release (1.5-fold; \( P<0.005 \)). Neutral endopeptidase contributes to bradykinin metabolism in heart failure patients maintained on angiotensin-converting enzyme inhibitor therapy. Our findings may explain some of the clinical effects of combined angiotensin-converting enzyme and neutral endopeptidase inhibition, including the greater vasodepressor effect observed with combined therapy when compared with angiotensin-converting enzyme inhibition alone. (Hypertension. 2004;44:913-918.)

Key Words: heart failure ■ angiotensin-converting enzyme ■ bradykinin ■ endothelium

Bradykinin is a potent endothelium-dependent vasodilator peptide released at sites of inflammation and coagulation. Apart from vasodilatation, it also stimulates endothelial release of the prolytic factor tissue-type plasminogen activator (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.1,2 Removal of the C-terminal arginine (t-PA), and these effects are mediated by the constitutively expressed B2 receptor.
upregulated in heart failure patients, and in the presence of ACE inhibition, the contribution of NEP to bradykinin metabolism is increased. NEP inhibition potentiates the half life of bradykinin and augments bradykinin-mediated vasodilatation in vitro. It is not known whether NEP inhibition augments the half life of Lys-des-Arg<sup>9</sup>-bradykinin. Indeed, it has been suggested that the Phe<sup>8</sup> residue may protect B1 NEP inhibition on systemic hemodynamics are variable, clinical improvements have been reported during NEP inhibition in heart failure patients.

Coadministration of ACE and NEP inhibitors may confer additional therapeutic efficacy. Combined ACE and NEP inhibition attenuates bradykinin degradation more effectively than either enzyme alone, and in animal models, improves cardiac remodeling and survival to a greater extent than isolated ACE inhibition. These cardioprotective effects are lost in transgenic mice lacking the B<sub>2</sub> kinin receptor. In man, combined ACE and NEP inhibition reduces blood pressure to a greater extent than inhibition of either enzyme alone and is associated with symptomatic and hemodynamic improvements in heart failure patients. The hypothesis that combined ACE and NEP inhibition may improve symptoms and survival in heart failure patients to a greater extent than ACE inhibition alone has been evaluated recently in a large-scale clinical trial (Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events [OVERTURE]).

We demonstrated previously that chronic ACE inhibition potentiates bradykinin-mediated vasodilatation and endothelial release of t-PA in forearm circulation of heart failure patients. The aims of this study were to investigate whether local NEP inhibition augments the vascular actions of bradykinin and to examine the effects of B<sub>2</sub> receptor agonism in heart failure patients maintained on long-term ACE inhibitor therapy.

**Methods**

**Patients**

Ten patients with symptomatic heart failure and echocardiographic evidence of left ventricular systolic dysfunction attended, fasted at 9 AM on 2 occasions at least 2 weeks apart. The protocol was performed with the approval of the local ethics committee, in accordance with the Declaration of Helsinki and with the written informed consent of each patient. Patients were maintained on maximally tolerated ACE inhibitor therapy (ramipril 10 mg daily [n = 4] and 5 mg daily [n = 3]; enalapril 20 mg daily [n = 1] and 10 mg daily [n = 1]; and lisinopril 40 mg daily [n = 1]) for at least 6 months before recruitment. On the morning of each visit, ACE inhibitor therapy was administered at 8 AM and diuretics were withheld.

**Measurements**

Bilateral forearm blood flow was measured using venous occlusion plethysmography. Heart rate and blood pressure were recorded in the noninfused arm using a semiautomated noninvasive oscillometric sphygmomanometer (Takeda UA 751; Takeda) at baseline and in the final minute of each drug infusion period, after forearm blood flow measurements and venous sampling.

Venous cannulae (17-gauge) were inserted bilaterally into a large antecubital vein. Throughout each study, 10 mL of blood was collected from each arm into acidified buffered citrate (Biopool Stablityle; Umeå; for t-PA assays) and citrate (Monovette, Sarstedt; Numbrecht; for plasminogen activator inhibitor-1 [PAI-1] assays). Platelet-free plasma was prepared as described previously and stored at −80°C before assay. Plasma concentrations of t-PA and PAI-1 antigen were determined using an ELISA, t-PA activity using a photometric method, and ACE activity using colorimetric spectrophotometry (reference range 8 to 55 U/L; Sigma).

**Study Protocol**

A 27-gauge steel needle was inserted into the brachial artery of the nondominant arm. After 15 minutes equilibration with an infusion of 0.9% saline, patients were randomized to receive an intrabrachial infusion of thiorphan (30 nmol/min; Clinalfa AG) or saline placebo for 3 hours. The dose of thiorphan was chosen based on previous forearm studies to achieve a local plasma concentration >10-fold the IC<sub>50</sub> of thiorphan for NEP in vitro. Thorphan or placebo was coinfused with bradykinin (30, 100, and 300 pmol/min; Clinalfa AG), Lys-des-Arg<sup>9</sup>-bradykinin (1, 3, and 10 nmol/min; Clinalfa AG), atrial natriuretic peptide (10, 30, and 100 pmol/min; Clinalfa AG), and sodium nitroprusside (2, 4, and 8 μg/min; David Bull Laboratories) for 10 minutes at each dose. There was a 20-minute washout infusion of 0.9% saline between compounds. The order of infusion was randomized between patients but was maintained for both visits. The doses of Lys-des-Arg<sup>9</sup>-bradykinin were chosen based on binding affinity data and the hypotensive dose response in rodents and nonhuman primates. The combined rate of infusion remained constant throughout each study at 1 mL/min.

**Data Analysis and Statistics**

Plethysmographic data were extracted from Chart data files, and forearm blood flow was calculated as described previously. Estimated net release of t-PA antigen and activity were calculated as the product of infused forearm plasma flow and the t-PA concentration difference between the infused and noninfused arms. Statistical analyses were performed using ANOVA or, where appropriate, paired t tests.

**Results**

There were no significant differences in heart rate, blood pressure, or baseline forearm blood flow during or between study days (Table 1). Consistent with previous studies, 1 subject developed transient upper limb edema with 300 pmol/min of bradykinin that rapidly resolved on cessation of the infusion. There were no other reported side effects.

**Plasma ACE Activity**

Baseline plasma ACE activity was similar between thiorphan and placebo study visits (12.3±2.6 versus 9.7±1.5 U/mL, respectively; P = 0.7). Compared with baseline, there were no significant differences in plasma ACE activity measured after 90 minutes of thiorphan (10.7±2.5 U/mL; P = 0.3) or placebo (10.2±1.7 U/mL; P = 0.3) cofusion.

**Forearm Blood Flow Responses**

Bradykinin, atrial natriuretic peptide, and sodium nitroprusside caused dose-dependent increases in forearm blood flow in all studies (P < 0.0001 for all; Figure 1). Forearm blood flow did not change during Lys-des-Arg<sup>9</sup>-bradykinin infusion (Figure 1). Compared with saline placebo, cofusion of thiorphan augmented forearm vasodilatation to bradykinin (P < 0.0001; Figure 1) but not atrial natriuretic peptide or sodium nitroprusside.

**Plasma Fibrinolytic Factors**

There were no significant differences in baseline plasma t-PA antigen (10.8±0.9 versus 9.8±0.9 ng/mL), t-PA activity...
TABLE 1. Patient Characteristics and Baseline Hemodynamics (n=10)

<table>
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<tr>
<th>Variables</th>
<th>All Patients (n=10)</th>
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<td>Baseline forearm blood flow</td>
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<tr>
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Data are expressed as No. of patients or mean±SEM.

Mean arterial pressure was calculated as diastolic pressure plus one third pulse pressure.

IHD indicates ischemic heart disease; DCM, idiopathic dilated cardiomyopathy; NYHA, New York Heart Association; LVEDD, left ventricular end diastolic diameter.

(0.1±0.1 versus 0.4±0.1), or PAI-1 antigen concentrations (37.3±3.7 versus 38.4±6.2 ng/mL) between thiorphan and placebo, respectively. Bradykinin caused a dose-dependent increase in plasma t-PA antigen and activity concentrations in the infused arm (P<0.001 for all; Table 2) and the net release of t-PA antigen and activity in all studies (P<0.005 for all; Figure 2). Compared with placebo, thiorphan augmented the increase in plasma t-PA activity concentration (21.9±2.4 versus 24.8±2.6 IU/mL, respectively at bradykinin, 300 pmol/min; P<0.05; Table 2) in the infused arm and the net release of t-PA antigen (157±46 versus 233±46 ng per 100 mL/min, respectively at bradykinin, 300 pmol/min; P<0.005; Figure 2) and activity (155±22 versus 244±51 ng per 100 mL/min, respectively at bradykinin, 300 pmol/min; P<0.005; Figure 2). There was a trend toward an increase in t-PA antigen in the infused arm during thiorphan infusion compared with placebo (32.5±3.4 versus 36.5±4.2 ng/mL, respectively at bradykinin, 300 pmol/min; P=0.058; Table 2). Because of systemic overspill, bradykinin increased plasma t-PA antigen and activity concentrations in the noninfused arm (P<0.01 and P<0.05, respectively; Table 2) that, for t-PA activity, was greater during thiorphan infusion (P<0.05; Table 2). There were no significant changes in t-PA antigen, activity, or net t-PA release during infusion of atrial natriuretic peptide or Lys-des-Arg⁸-bradykinin.

There was a significant reduction in plasma PAI-1 antigen concentrations in the infused arm during bradykinin coinfusion with thiorphan (baseline 33.3±3.5 versus 29.4±3.4 ng/mL at bradykinin, 300 pmol/min; P<0.05) but not placebo. There were no significant changes in PAI-1 antigen concentration during infusion of Lys-des-Arg⁸-bradykinin or atrial natriuretic peptide.

**Discussion**

For the first time, we demonstrated that acute local NEP inhibition augments bradykinin-mediated endothelium-dependent vasodilation and endogenous t-PA release in heart failure patients maintained on chronic ACE inhibitor therapy. In addition, this is the first clinical study to show that the B₁ receptor does not mediate vasodilatation or endothelial t-PA release in these patients. Our findings support the hypothesis that bradykinin may contribute to the systemic hemodynamic differences observed between combined ACE and NEP inhibition, and ACE inhibition alone.

**Clinical Implications**

There is now substantial evidence that bradykinin contributes to the systemic hemodynamic and anti-ischemic effects of...
ACE inhibitor therapy. Our findings suggest that bradykinin-mediated vasodilatation may contribute to the greater vasodepressor actions demonstrated with combined ACE and NEP inhibition compared with isolated ACE inhibition. Moreover, despite the marked increase in bradykinin-induced t-PA release by ACE inhibition alone, additional NEP inhibition causes further substantial augmentation of acute t-PA release. Together, these hemodynamic and profibrinolytic effects would be expected to have important therapeutic consequences. However, in the recent OVERTURE trial of heart failure patients, treatment with omapatrilat, a combined ACE and NEP inhibitor, failed to reduce all-cause mortality when compared with enalapril, although it did reduce the combined secondary end point of cardiovascular death and hospitalization. Post hoc analysis redefining end points according to the Studies of Left Ventricular Dysfunction (SOLVD) criteria suggested that omapatrilat may be more effective at preventing cardiovascular events than enalapril, but that the additional benefit was substantially smaller than anticipated. This may, in part, reflect the shorter duration of NEP inhibition compared with ACE inhibition with omapatrilat. Given our findings, pharmacological strategies offering a more balanced and prolonged duration of combined ACE and NEP inhibition may confer greater cardiovascular benefits.

Augmentation of bradykinin-mediated vasodilatation within the kidney may also contribute to the greater increase in renal blood flow observed with combined ACE and NEP inhibition than ACE inhibition alone. As a result, it has been suggested that combined ACE and NEP inhibition may afford greater renal protection than ACE inhibition alone. However, it should be noted that potentiating the vascular actions of kinins may have detrimental effects. Bradykinin has been implicated in the pathogenesis of ACE inhibitor–mediated angioedema. An even greater incidence of angioedema has been reported after treatment with combined ACE and NEP inhibition. Our findings are consistent with the suggestion that bradykinin may contribute to this rare but potentially life-threatening side effect.

**Role of B₁ Receptor**

We demonstrated that intra-arterial Lys-des-Arg⁵-bradykinin, a potent and highly selective agonist at the human B₁ kinin receptor, has no effect on blood flow or endothelial t-PA release. However, bradykinin-induced t-PA release by ACE inhibition alone, has no effect on blood flow or endothelial t-PA release. However, bradykinin-mediated vasodilatation may contribute to the greater increase in renal blood flow observed with combined ACE and NEP inhibition than ACE inhibition alone. As a result, it has been suggested that combined ACE and NEP inhibition may afford greater renal protection than ACE inhibition alone. However, it should be noted that potentiating the vascular actions of kinins may have detrimental effects. Bradykinin has been implicated in the pathogenesis of ACE inhibitor–mediated angioedema. An even greater incidence of angioedema has been reported after treatment with combined ACE and NEP inhibition. Our findings are consistent with the suggestion that bradykinin may contribute to this rare but potentially life-threatening side effect.

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release in the forearm circulation of heart failure patients maintained on long-term ACE inhibitor therapy. This is in contrast to our previous findings that combined B1 and B2 receptor blockade, but not isolated selective blockade of the B2 receptor causes vasoconstriction in heart failure patients treated with ACE inhibition.7 We infused Lys-des-Arg9-bradykinin at a dose that would achieve a local plasma concentration 20× greater than those shown previously to produce 50% of the maximal vasomotor response in human33 and animal studies,34,35 and therefore, our findings are unlikely to reflect an inadequate dose. Additional clinical studies using a selective B1 kinin receptor antagonist are now required to more fully investigate the role of the vascular B1 receptor in man.

Atrial Natriuretic Peptide and Neutral Endopeptidase Consistent with previous work demonstrating an impaired forearm vasodilator response to atrial natriuretic peptide in heart failure patients,32–42 atrial natriuretic peptide caused a modest dose-dependent increase in forearm blood flow that was not augmented by local NEP inhibition. Although suppression of PAI-1 expression in endothelial cells has been reported in vitro,43 we report for the first time that intrarterial atrial natriuretic peptide does not directly alter either plasma PAI-1 or t-PA antigen concentrations in vivo in man.

Local NEP inhibition did not potentiate atrial natriuretic peptide–mediated forearm vasodilatation, in keeping with previous data demonstrating that intrabrachial thiorphan (30 nmol/min) does not increase endogenous plasma atrial natriuretic peptide concentrations in human forearm circulation.30 At first, this may appear surprising given that systemic NEP inhibition augments plasma atrial natriuretic peptide concentrations in heart failure patients.21 However, it is likely to reflect differences in the rate of clearance of atrial natriuretic peptide and bradykinin from forearm circulation. The half life of atrial natriuretic peptide (∼5 minutes) is greater than that of bradykinin (∼15 seconds). Assuming a transit time of the forearm vascular bed of ∼30 seconds, NEP inhibition with intrabrachial thiorphan is unlikely to result in sufficient local accumulation of atrial natriuretic peptide to augment forearm vasomotor responses. Moreover, the natriuretic peptide C receptor contributes equally to the clearance of plasma atrial natriuretic peptide, and this pathway is unaffected by NEP inhibition.

Figure 2. Effect of thiorphan (●) and placebo (○) on net tissue plasminogen activator (t-PA) antigen (top) and activity (bottom) release during intra-arterial infusion of bradykinin and atrial natriuretic peptide (●P<0.005, ANOVA dose response; †P<0.005, ANOVA thiorphan vs placebo).

Study Limitation Although selective for NEP, thiorphan may cause some inhibition of ACE activity, and theoretically, our findings could represent further inhibition of ACE activity. Thiorphan exists as 2 enantiomers that, although equipotent for NEP inhibition, have differing potencies against ACE: selectivity of NEP compared with ACE inhibition of ∼50-fold for S-thiorphan and 200-fold for R-thiorphan.11 The preparation of thiorphan used in this study contains equal proportions of both isomers,11 We do not believe the effects of thiorphan were mediated through additional inhibition of ACE activity because local thiorphan infusion did not alter plasma ACE activity. Moreover, in a previous study30 using the same dose of thiorphan, there were no effects on plasma angiotensin II concentrations in forearm circulation of healthy volunteers treated acutely with enalapril. However, we acknowledge that ACE inhibition by maximally tolerated doses of an ACE inhibitor may be incomplete,44 and we cannot completely exclude a contribution of additional ACE inhibition to our study findings.

Perspectives We have demonstrated that local NEP inhibition augments bradykinin-mediated vasodilatation and endothelial t-PA release in heart failure patients maintained on long-term ACE inhibitor therapy. Using a potent B1 receptor agonist, we have shown that isolated stimulation of the B1 kinin receptor does not cause vasodilatation or endothelial t-PA release in man. These findings confirm that NEP contributes to bradykinin metabolism in heart failure patients and suggest that potentiation of the vascular and profibrinolytic actions of bradykinin may explain some of the observed effects in recent clinical trials of combined ACE and NEP inhibitor therapy.

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References
22. Dumoulin MJ, Adam A, Rouleau JL, Lamontagne D. Comparison of a


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