Angiotensin-Converting Enzyme Inhibition Increases Human Vascular Tissue-Type Plasminogen Activator Release Through Endogenous Bradykinin

Mias Pretorius, MBChB, DA (SA); David Rosenbaum, MD; Douglas E. Vaughan, MD; Nancy J. Brown, MD

Background—Angiotensin-converting enzyme (ACE) inhibition potentiates the tissue-type plasminogen activator (t-PA) response to exogenous bradykinin. This study tested the hypothesis that ACE inhibition increases endothelial t-PA release through endogenous bradykinin.

Methods and Results—We measured the effect of intra-arterial enalaprilat (5 μg/min) on forearm blood flow (FBF) and net t-PA release before and during intra-arterial infusion of bradykinin (25 to 400 ng/min) and methacholine (3.2 to 12.8 μg/min) in 24 smokers pretreated with bradykinin receptor antagonist HOE 140 (100 μg/kg intravenously) or vehicle. There was no specific effect of HOE 140 on FBF or forearm vascular resistance (FVR, 29.9±3.6 versus 29.7±3.6 mm Hg · mL⁻¹ · min⁻¹ · 100 mL⁻¹ after vehicle and HOE 140, respectively, P=0.956 between groups). Resting FVR decreased during enalaprilat compared with vehicle or HOE 140, but not compared with baseline, and the effect was similar in the 2 groups (22.0±2.7 and 24.1±2.9 mm Hg · mL⁻¹ · min⁻¹ · 100 mL⁻¹, respectively, P=0.610). In contrast, enalaprilat significantly increased resting net t-PA release (from 0.6±0.4 to 1.7±0.6 ng · min⁻¹ · 100 mL⁻¹, P=0.002); this effect was abolished by HOE 140 (0.1±0.3 ng · min⁻¹ · 100 mL⁻¹, P=0.036 versus enalaprilat alone). Enalaprilat increased the effect of exogenous bradykinin on FBF 60% (from 17.5±2.5 to 28.1±4.0 mL · min⁻¹ · 100 mL⁻¹ during 100 ng/min bradykinin, P=0.001) and on t-PA release 14-fold (from 21.2±5.9 to 317.4±118.9 ng · min⁻¹ · 100 mL⁻¹, P=0.024). Enalaprilat increased the t-PA response to bradykinin to a greater extent than the FBF response, shifting the relationship between net t-PA release and FBF (P=0.005). HOE 140 blocked these effects. There was no effect of enalaprilat or HOE 140 on the FBF or t-PA response to methacholine.

Conclusion—ACE inhibition increases constitutive endothelial t-PA release through endogenous bradykinin. (Circulation. 2003;107:579-585.)

Key Words: angiotensin • bradykinin • fibrinolysis

A cute rupture of a coronary atheromatous plaque and the subsequent coronary thrombosis play a critical role in the pathogenesis of myocardial infarction (MI) and sudden cardiac death.¹ The endogenous fibrinolytic system limits thrombosis and promotes reperfusion.² Impairment of endothelial fibrinolytic capacity in patients with risk factors for coronary artery disease may contribute to morbidity and mortality as a result of MI in these patients.³

Angiotensin-converting enzyme (ACE) inhibitors improve endothelial function as measured by the vasodilator response to endothelium-dependent agonists.⁴ In addition, ACE inhibitors have been shown to improve fibrinolytic balance by decreasing circulating concentrations of plasminogen activator inhibitor-1 (PAI-1), the major physiological inhibitor of tissue type plasminogen activator (t-PA).⁵⁻⁷ The findings in several studies that ACE inhibitors decrease PAI-1 without decreasing t-PA antigen⁶⁻⁸⁻⁹ (which reflects both free active t-PA and t-PA bound to PAI-1 and other inhibitors¹⁰) suggested that ACE inhibitors may increase t-PA release through effects on endogenous bradykinin (BK). Indeed, there is substantial evidence that exogenous BK stimulates t-PA release from the human forearm vasculature¹¹ and that ACE inhibition potentiates this effect in the peripheral¹² and coronary vasculature.¹³ Although endogenous BK contributes to the hemodynamic effects of acute ACE inhibition,¹⁴ the analogous physiological role in t-PA release has not been explored. Therefore, we tested the hypothesis that ACE inhibition stimulates endothelial t-PA release through an effect on endogenous BK.
Methods

Subjects
Twenty-four smokers were studied. Subjects were defined as smokers if they smoked 5 to 25 cigarettes per day. We have previously determined that bradykinin-stimulated t-PA release but not vasodilation is attenuated in otherwise healthy smokers compared with nonsmokers. After written informed consent was obtained, all subjects underwent a complete history and physical examination. Subjects with cardiovascular, renal, pulmonary, endocrine, or hematological disease were excluded. All subjects were within 30% of ideal body weight. Pregnancy was excluded in women of childbearing potential by measurement of urine β-human chorionic gonadotropin. Subjects with fasting cholesterol >5.17 mmol/L (200 mg/dL) were excluded.

Experimental Protocol
The study protocol (Figure 1) was approved by the Vanderbilt University Institutional Review Board and conducted according to the Declaration of Helsinki. Subjects were studied in the supine position after overnight fast. An intravenous catheter was placed in the antecubital vein in both arms. After subdermal administration of 1% lidocaine, an 18 gauge polyurethane catheter (Cook Inc) was inserted into the brachial artery of the nondominant arm. Catheter patency was maintained by infusion of 1 mL/min 5% dextrose in water. After catheter placement, subjects rested 30 minutes before baseline measurements. After measurement of basal forearm blood flow (FBF) and blood sampling, graded doses of sodium nitroprusside (SNP), methacholine (Mch), and BK were infused in random order. SNP was infused at 1.6, 3.2, and 6.4 μg/min; Mch at 3.2, 6.4, and 12.8 μg/min; and BK at 100, 200, and 400 ng/min. BK, at these doses, causes vasodilation in part through a nitric oxide synthase (NOS)–dependent pathway. In contrast, Rongen et al reported that Mch induces vasodilation through a NOS-independent pathway. Similarly, in a pilot study in 6 subjects, we found no effect of the NOS inhibitor Nω-nonmonomethyl-arginine (L-NMMA, 4 μmol/min) on Mch-mediated vasodilation (maximal FBF response to Mch 28.0±3.8 mL·min⁻¹·100 mL⁻¹ versus 26.8±4.5 mL·min⁻¹·100 mL⁻¹ in the absence and presence of L-NMMA). Nevertheless, we chose Mch as the endothelium-dependent control because it has been shown to stimulate t-PA release. Each dose of agonist was infused for 5 minutes and FBF was measured during the last 2 minutes.

Thirty minutes after administration of these three drugs, baseline measurements were repeated and subjects were randomized to receive vehicle (normal saline) or 100 μg/kg HOE 140 intravenously over 1 hour. This dose of HOE 140 has no effect on systemic blood pressure or heart rate, but blocks both the vasodilator and t-PA responses to infused BK for at least 3 hours. FBF measurements and arterial and venous sampling were repeated after HOE 140. At this time, enalaprilat was infused in the brachial artery at a rate of 5 μg/min. This dose blocks conversion of Ang I to Ang II and potentiates the vasodilator effect of BK in the forearm. During enalaprilat, baseline measurements and infusions of Mch and BK were repeated. Three subjects complained of headache during Mch infusion. Two of the first 9 subjects developed transient arm swelling after infusion of BK in the presence of enalaprilat; therefore the dose of BK infused during enalaprilat was reduced to 25, 50, and 100 ng/min in the remaining 15 (7 vehicle-treated, 8 HOE 140-treated) subjects.

Forearm Perfusion Measurements, Blood Sampling, and Biochemical Assays
FBF was measured by silastic-in-mercury strain-gauge plethysmography, as described previously. After measurement of FBF, simultaneous arterial and venous samples were obtained from the infused arm before and after each dose of Mch and BK. Because SNP does not increase net t-PA release, no blood was drawn during SNP. Infusions were interrupted during arterial sampling. Blood samples were collected on ice, centrifuged immediately, and plasma was stored at −70°C until assay. Blood for measurement of PAI-1 and t-PA was collected in tubes containing 0.105 mol/L acidified sodium citrate, and antigen levels were determined with a 2-site ELISA (Biopool AB). t-PA activity was not measured, as we have demonstrated previously that active t-PA increases with t-PA release.

Arteriovenous concentration gradients were calculated by subtracting the plasma level measured in simultaneously collected venous and arterial blood. Forearm plasma flow was calculated from the FBF and arterial hematocrit corrected for 1% trapped plasma.

Figure 1. Study protocol. The inset demonstrates the effects of the pharmacological agents studied on the pathways involved in endothelium-dependent vasodilation and t-PA release. PLC indicates phospholipase C; IP₃, inositol(1,4,5)triphosphate; PLA₂, phospholipase A₂; NOS, nitric oxide synthase; PG₁₂, prostacyclin; EDHF, endothelium-derived hyperpolarizing factor; SNP, sodium nitroprusside; MCH, methacholine; and BK, bradykinin.
TABLE 1. Subject Characteristics

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Results

Statistical Analysis
Data are presented as mean ± SEM. Categorical data were compared with χ² or Fischer’s exact tests, as appropriate. The effect of drugs were compared with a general linear model-repeated measures analysis of variance (ANOVA) in which the between-subject variables were treatment group (vehicle or HOE 140), sex, and ethnicity, and the within-subjects variables were the dose of BK or MCh and the within-subjects variables were the dose of BK or MCh and the within-subjects variables were the dose of BK or MCh and the presence or absence of enalaprilat. Although safety considerations required us to reduce the BK dose administered during enalaprilat, all subjects received the 100-ng/min dose in both the presence and absence of enalaprilat; therefore, the effect of enalaprilat on the FBF or t-PA response to this dose was determined with a paired t test. A 2-tailed probability value < 0.05 was considered statistically significant. All analyses were performed with the statistical package SPSS for Windows (Version 11.0.0, SPSS, Chicago).

Effect of HOE 140 and Enalaprilat on Resting FBF and Net t-PA Release
There was no effect of vehicle or HOE 140 on MAP (Table 2). MAP decreased during intra-arterial infusion of enalaprilat in the vehicle-treated group (P = 0.026) but not in the HOE 140-treated group (P = 0.422). Resting FVR increased after infusion of either vehicle or HOE 140 (P < 0.05 versus baseline for either treatment, Figure 2 and Table 2). However, FVR remained similar in the vehicle- and HOE 140-treated groups (P = 0.956 between groups), suggesting an effect of time or vehicle, rather than a specific effect of BK receptor antagonism on FVR. Resting FVR decreased after intra-arterial infusion of enalaprilat (Figure 2 and Table 2); again the effect was similar in the vehicle- and HOE 140-treated groups (P = 0.610).

There was no effect of either vehicle or HOE 140 on net t-PA release (Table 2 and Figure 2). However, intra-arterial infusion of enalaprilat significantly increased constitutive t-PA release across the forearm in the vehicle-treated group (P = 0.002). Although there was no effect of sex alone (P = 0.720) on t-PA release, there was a significant interactive effect of ACE inhibition and sex (P = 0.028) on resting t-PA release such that ACE inhibition increased resting t-PA release in women (from 0.2 ± 0.4 to 2.5 ± 0.8 ng·min⁻¹·100 mL⁻¹, P = 0.03) but not men (from 1.0 ± 0.8 to 0.5 ± 0.8 ng·min⁻¹·100 mL⁻¹, P = 0.878). Pretreatment with HOE 140 abolished the effect of enalaprilat on net t-PA release. This effect was seen in women (net t-PA release 0.5 ± 0.5 and 0.5 ± 0.1 ng·min⁻¹·100 mL⁻¹ before and after HOE 140 plus enalaprilat, respectively) as well as men (net t-PA release 0.5 ± 1.0 and 0.0 ± 0.4 ng·min⁻¹·100 mL⁻¹ before and after HOE 140 plus enalaprilat, respectively). Thus, constitutive t-PA release was significantly lower after HOE 140 plus enalaprilat than after enalaprilat alone (P = 0.036).

Effect of HOE 140 and Enalaprilat on FBF Response to BK and MCh
There was no effect of intra-arterial infusion of BK alone (P = 0.878) or in the presence of enalaprilat (P = 0.796) on MAP. BK significantly increased FBF in a dose-dependent fashion (P < 0.001 Figures 3A and B). Administration of enalaprilat significantly increased the FBF response to BK (P = 0.024) in the vehicle-treated group. Hence, the FBF in response to 100 ng/min BK was 60% higher during enalaprilat (28.1 ± 4.0 mL·min⁻¹·100 mL⁻¹ versus 17.6 ± 2.5 mL·min⁻¹·100 mL⁻¹, P = 0.001). Pretreatment with the BK receptor antagonist HOE 140 not only blocked the effect of enalaprilat, but also decreased the FBF response to BK to less than that measured in the absence of enalaprilat (8.9 ± 1.5 mL·min⁻¹·100 mL⁻¹ during enalaprilat plus HOE 140 versus 17.6 ± 2.0 mL·min⁻¹·100 mL⁻¹ in response to 100 ng/min BK alone, P = 0.001).

There was no effect of MCh alone (P = 0.512) or in the presence of enalaprilat (P = 0.519 in the vehicle-treated group) on MAP. MCh significantly increased FBF in a dose-dependent manner, from 4.4 ± 0.5 to 36.1 ± 2.5 mL·min⁻¹·100 mL⁻¹ (P < 0.001). Enalaprilat (P = 0.112) and
HOE 140 ($P=0.763$) did not alter the FBF response to MCh. The FBF response to MCh was significantly greater than the FBF response to BK in the presence ($P=0.014$ for the effect of MCh versus BK during enalaprilat in the vehicle-treated group) and absence ($P<0.001$) of ACE inhibition. SNP also increased FBF, from $4.3\pm0.3$ to $21.6\pm1.9$ mL·min$^{-1}$·$100$ mL$^{-1}$ ($P=0.001$).

**Effect of HOE 140 and Enalaprilat on Net t-PA Response to BK and MCh**

BK significantly increased net t-PA release from $0.5\pm0.4$ to $37.8\pm9.4$ ng·min$^{-1}$·$100$ mL$^{-1}$ at the highest dose ($P=0.005$, Figures 3C and D). Administration of enalaprilat significantly increased the t-PA response to BK ($P=0.001$) in the vehicle-treated group and there was no interactive effect with sex. Thus, net t-PA release in response to 100 ng/min BK was 14-fold higher during enalaprilat ($317.4\pm118.9$ versus $21.2\pm7.9$ ng·min$^{-1}$·$100$ mL$^{-1}$, $P=0.024$). HOE 140 blocked this effect of ACE inhibition ($P<0.001$ for an ACEI × HOE 140 interaction), such that the t-PA response to BK in the presence of enalaprilat plus HOE 140 was similar to that in the absence of enalaprilat ($P=0.422$). In the 12 subjects who were randomized to vehicle, ACE inhibition shifted the relation-
ship between FBF and t-PA release in response to the maximal dose of BK ($P=0.005$ for the difference between the slopes, Figure 4), confirming that ACE inhibition increased the t-PA response to BK to a greater extent than the FBF response.

MCh significantly increased net t-PA release in a dose-dependent fashion, from 0.3±0.4 to 22.1±8.5 ng · min$^{-1}$ · 100 mL$^{-1}$, $P=0.017$. There was no effect of either HOE 140 ($P=0.443$) or enalaprilat ($P=0.296$) on the t-PA response to MCh. The t-PA response to BK was significantly greater than the response to MCh during ACE inhibition ($P=0.012$) and tended to be greater than the response to MCh even in the absence of ACE inhibition ($P=0.074$).

There was no effect of enalaprilat ($P=0.934$), HOE 140 ($P=0.467$), BK ($P=0.771$), or MCh ($P=0.486$) on net PAI-1 extraction across the forearm (data not shown).

Discussion

This study examines the contribution of endogenous BK to the effect of acute ACE inhibition on human forearm vasodilation and fibrinolysis in a group of subjects with impaired fibrinolytic function, smokers. The study confirms prior studies with HOE 140 indicating that endogenous BK does not contribute to resting vascular tone. However, this is the first study to demonstrate that ACE inhibition increases constitutive forearm endothelial t-PA release through an effect on endogenous BK.

To explore the possible mechanism(s) through which ACE inhibition enhances endothelial t-PA release, we also compared the effect of ACE inhibition on the vasodilator and t-PA responses to exogenous BK and to the muscarinic agonist MCh. Although BK and MCh act through unique receptors (B$_2$ and M$_3$, respectively), both agonists act through G$\alpha_q$ to activate phospholipase C and stimulate the production of inositol triphosphate. Both BK and MCH stimulate the release of endothelium-derived hyperpolarizing factor. As reported previously, MCh significantly increased t-PA release, as well as FBF. Over the doses given, MCh caused greater vasodilation and less t-PA release than did BK. The effect of MCh on endothelial t-PA release contrasts with a reported lack of effect of acetylcholine on endothelial t-PA release. The disparity may relate to the comparative decreased potency of acetylcholine and its susceptibility to cholinesterases.

Importantly, whereas enalaprilat potentiated the vasodilator response to BK, there was no effect of ACE inhibition on MCh-stimulated vasodilation. This contrasts data from Nakamura et al indicating that enalaprilat potentiates nitric oxide-mediated endothelium-dependent vasodilation in response to ACh. One explanation for the lack of effect of enalaprilat on MCh-mediated vasodilation is that MCh, unlike ACh, causes vasodilation through a NOS-independent pathway. ACE inhibition also potentiated the t-PA response to BK but not that to MCh in this study. Similarly, Labinjoh et al have reported that ACE inhibition enhances the t-PA response to exogenous BK but not to substance P. Taken together with the finding that HOE 140 abolished the effect of ACE inhibition on BK-stimulated t-PA release, the data suggest that ACE inhibition enhances vascular t-PA secretion through a BK B$_2$-receptor-specific pathway.

In this study, ACE inhibition enhanced endothelial fibrinolytic function to a greater extent than vasodilator function. For example, enalaprilat increased t-PA release via endogenous BK, even when given at a concentration that did not affect resting FBF. ACE inhibition potentiated the t-PA response to exogenous BK to a greater extent than the vasodilator effect (14-fold versus 60% at the 100-ng/min dose). Conversely, BK receptor antagonism reduced the vasodilator response to BK to a greater extent than the t-PA response.

The mechanism(s) whereby ACE inhibition differentially affected the vasodilator and fibrinolytic functions of the endothelium is not certain. ACE inhibitors potentiate the effects of BK not only by decreasing its degradation but also by enhancing sensitivity to BK at a receptor level. In addition, BK may stimulate vasodilation and t-PA release through multiple or different mechanisms. For instance, administration of the NOS inhibitor L-NMMA attenuates the forearm vasodilator response to BK, indicating that the NO contributes to BK-induced vasodilation. In contrast, L-NMMA alone or in combination with indomethacin does not affect the t-PA response to BK, suggesting that t-PA release is mediated through a NOS- and cyclooxygenase-independent pathway. In the present study, the finding that ACE inhibition altered the relationship between BK-stimulated FBF and t-PA release suggests the possibility that ACE inhibition potentiated t-PA release not only by decreasing BK degradation, but also by altering sensitivity to BK at a receptor or post-receptor level.

ACE inhibitors improve fibrinolytic balance in part by decreasing circulating concentrations of PAI-1. In the present study, although enalaprilat increased constitutive and BK-stimulated t-PA release there was no effect of intra-arterial enalaprilat or BK on net PAI-1 extraction. This relates to the fact that whereas endogenous and exogenous BK stimulate acute release of preformed t-PA from the vasculature, ACE inhibition decreases PAI-1 concentrations by preventing the effect of Ang II on PAI-1 expression, an effect that would not be seen over the short course of ACE inhibition in the present study. Indeed, Oshima et al have reported that...
ACE inhibition reduces circulating PAI-1 concentrations by 48 hours after initiation of treatment. Although this study explored the effect of ACE inhibition on endothelial fibrinolytic function of the forearm vasculature, Minai et al. have demonstrated that exogenous BK stimulates t-PA release from the coronary vasculature as well and that this effect is potentiated by ACE inhibition. In contrast to the present study, these investigators reported no effect of oral administration of enalapril on resting coronary t-PA release. Similarly, Labinjoh et al. did not note an effect of oral quinapril on estimated net t-PA release. However, neither group explored the contribution of endogenous BK using HOE 140. Although the discrepancy between the data of Minai et al. and the results of the present study about the effect of ACE inhibition on resting t-PA release raises the possibility that results obtained in the forearm vasculature may not be applicable to the coronary vasculature, other methodological differences should be considered. For example, intra-arterial infusion of enalaprilat in the present study may have resulted in greater local vascular ACE inhibition than could have been achieved after oral ACE inhibition in either the study by Minai et al. or that of Labinjoh et al. Additional studies are needed to determine whether the effect of ACE inhibition on constitutive endothelial t-PA release is either dose-dependent or vascular bed-specific.

A more important methodological difference between this study and prior studies relates to the inclusion of women. Minai et al. studied an older, predominantly (72%) male population and Labinjoh et al. studied males exclusively. An unexpected finding in this study was that ACE inhibition significantly increased net t-PA release only in the female subjects studied. The mechanism for this effect is not certain, but women appear to be more sensitive than men to other effects of ACE inhibitors that are potentially mediated by BK, including cough and angioedema. In the present study, all of the women studied were premenopausal or taking hormone replacement therapy. Estrogen has been shown to decrease ACE activity. In addition, estrogen upregulates BK receptors, activates potassium channels (which may in turn promote endothelium-dependent hyperpolarization), and sensitizes human coronary arteries to BK-mediated vasodilation. Although the present study was not powered to address sex differences in response to ACE inhibition, per se, and although we cannot exclude the possibility that an effect of ACE inhibition on constitutive t-PA release would have been seen in a larger group of men, studies are needed to further investigate the extent to which sex or estrogen modulate the effect of ACE inhibition on endothelial fibrinolytic function. The clinical relevance of these studies is underscored by recent data from the HOPE trial suggesting a favorable interactive effect of hormone replacement therapy and ACE inhibition on the risk of cardiovascular events.

In summary, this study demonstrates that ACE inhibition increases constitutive endothelial t-PA release through endogenous BK. This effect appears to be sex specific. In addition, ACE inhibition potentiates the effect of BK on the fibrinolytic function of the endothelium to a greater extent than the vasodilator function. The enhancement of local vascular endothelial t-PA release may contribute to the cardioprotective effects of ACE inhibitors.

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References


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