The fibrinolytic factor tissue plasminogen activator (t-PA) is a serine protease that regulates the degradation of intravascular fibrin and is released from the endothelium through the translocation of a dynamic intracellular storage pool (1,2). If endogenous fibrinolysis is to be effective then the rapid mobilization of t-PA from the endothelium is essential, because thrombus dissolution is much more effective if t-PA is incorporated during, rather than after, thrombus formation (3). The efficacy of plasminogen activation and fibrin degradation is further determined by the relative balance between the acute local release of t-PA and its subsequent inhibition through formation of complexes with the serpin plasminogen activator inhibitor type 1 (PAI-1). This dynamic aspect of endothelial function and fibrinolytic balance may be directly relevant to the pathogenesis of atherothrombosis, but only recently have robust methods to determine acute t-PA release been developed (4–7). Small areas of denudation and thrombus deposition are a common finding on the surface of atheromatous plaques (8,9) and are usually subclinical. However, in the presence of an imbalance in the coagulation or fibrinolytic systems, such microthrombi may propagate, ultimately leading to arterial occlusion (10). Bradykinin is released during the contact phase of coagulation, when high-molecular-weight kininogen is cleaved by kallikrein to produce a disulphide-linked light and heavy chain (11,12). Although an inflammatory mediator, bradykinin is also a potent endothelial cell stimulant that can induce the acute release of t-PA from the endothelium (7,13) through a B2 receptor mechanism (14). Thus, following activation of the intrinsic coagulation pathway, the liberation of bradykinin may represent an important negative feedback loop in which bradykinin—
induced t-PA release inhibits thrombus formation within the vascular lumen when localized endothelial denudation occurs. Furthermore, given that bradykinin-induced forearm vasodilatation is potentiated by angiotensin-converting enzyme (ACE) inhibition (15), such actions may be enhanced in the presence of ACE inhibition and may, in part, explain the anti-ischemic action of this therapy in vascular disease (16). However, although inferred, the potentiation of bradykinin-induced t-PA release by ACE inhibition has yet to be established. Therefore, the aim of the present study was to determine whether systemic ACE inhibition could augment acute local t-PA release from the endothelium in vivo in humans.

METHODS

Subjects. Eight healthy male nonsmokers, ages between 21 and 30 years, participated in the study, which was undertaken with the approval of the local research ethics committee, in accordance with the Declaration of Helsinki and with the written informed consent of each subject. Except for study medication, none of the subjects received vasoactive drugs in the week before each phase of the study, and all abstained from alcohol for 24 h and from food and caffeine-containing drinks for at least 4 h before each study. All studies were carried out in a quiet, temperature-controlled room maintained at 22°C to 24°C.

Measurements. FOREARM BLOOD FLOW AND HEMODYNAMICS. Blood flow was measured in both forearms by venous occlusion plethysmography using mercury-in-silastic strain gauges applied to the widest part of the forearm as previously described (17). Analogue voltage output from an EC-4 Strain Gauge Plethysmograph (D.E. Hokanson Inc., Bellevue, Washington) was processed by a MacLab analogue-to-digital converter and Chart v3.3.8 software (AD Instruments Ltd., Castle Hill, Australia) and recorded using a MacLab data acquisition system (AD Instruments Ltd., Kings Langley, United Kingdom) as previously described (5,7,19). Estimated net release of t-PA activity and antigen was previously described (5,7,19). Estimated net release of t-PA activity and antigen was previously defined as the product of the infused forearm plasma flow (based on the mean hematocrit and the infused forearm blood flow) and the concentration difference between the infused and noninfused arms. Data were examined, where appropriate, by analysis of variance (ANOVA) with repeated measures and two-tailed paired Student t test using Excel v5.0 (Microsoft). All results are expressed as mean ± standard error of the mean. Statistical significance was taken at the 5% level.

Plasma t-PA and PAI-1 antigen and activity concentrations were determined using enzyme-linked immunosorbent assays and a photometric method as previously described (5,19). Plasma ACE activity was determined using a spectrophotometric technique (Sigma Chemical Corporation, St. Louis, Missouri) (20). Hematocrit was determined by capillary tube centrifugation of blood anticoagulated by ethylene diamine tetraacetic acid.

Study design. Subjects attended on each of three study days, two weeks apart, having received matched placebo, quinapril 40 mg and losartan 50 mg (an angiotensin II type 1 (AT1) receptor antagonist) once daily for the seven days before attendance. The subjects received each of the medications in a double-blind randomized crossover design. On each study day, the final dose of placebo, quinapril or losartan was taken at 08:00 hr. Six hours later, the subjects rested recumbent, and strain gauges and cuffs were applied to the forearms. The brachial artery of the nondominant arm was cannulated with a 27-standard gauge stainless steel needle (Cooper’s Needle Works Ltd., Birmingham, United Kingdom) under 1% lidocaine (Xylocaine: Astra Pharmaceuticals Ltd., Kings Langley, United Kingdom) local anesthesia. The total rate of intra-arterial infusions was maintained constant at 1 ml/min and forearm blood flow was measured every 10 min throughout all studies. Intrabrachial infusions of substance P (Clinalfa AG, Läufelfingen, Switzerland; endothelium-dependent vasodilator) at 2, 4 and 8 pmol/min (5,19), sodium nitroprusside (David Bull Laboratories, Warwick, United Kingdom; endothelium-independent vasodilator) at 2, 4 and 8 μg/min (5,7) and bradykinin (Clinalfa AG; endothelium-dependent vasodilator) at 100, 300 and 1,000 pmol/min were given for 10 min at each dose in that order. Saline was infused for 30 min before the substance P, sodium nitroprusside and bradykinin infusions.

Data analysis and statistics. Plethysmographic data were extracted from the Chart data files. Forearm blood flows were calculated for individual venous occlusion cuffs inflations by a template spreadsheet (Excel v5.0; Microsoft Corporation, Cambridge, Massachusetts) as previously described (5,7,19). Estimated net release of t-PA activity and antigen was previously defined (5,7,19) as the product of the infused forearm plasma flow (based on the mean hematocrit and the infused forearm blood flow) and the concentration difference between the infused and noninfused arms. Data were examined, where appropriate, by analysis of variance (ANOVA) with repeated measures and two-tailed paired Student t test using Excel v5.0 (Microsoft). All results are expressed as mean ± standard error of the mean. Statistical significance was taken at the 5% level.
RESULTS

Oral and intra-arterial drug administrations were well tolerated without significant adverse effects. Consistent with previous studies (7), transient patchy flushing and skin edema of the infused arm occurred with bradykinin infusion at doses ≥300 pmol/min. There were no significant changes in heart rate, blood pressure and noninfused forearm blood flow (data on file) during or between study days. In comparison to placebo and losartan, plasma ACE activity was suppressed during quinapril administration (14.1 ± 0.8 IU/ml, 17.9 ± 2.4 IU/ml and 7.6 ± 1.6 IU/ml respectively: p < 0.004, ANOVA).

Forearm blood flow responses. Substance P, sodium nitroprusside and bradykinin produced dose-dependent forearm vasodilatation during each study visit (one-way ANOVA: p < 0.001 for all) (Fig. 1). There were no significant differences between the magnitude of the forearm blood flow responses during placebo, quinapril or losartan administration (two-way ANOVA: p = ns for treatment group, p < 0.001 for forearm blood flow response, p = ns for interaction). There was a modest trend for bradykinin-induced vasodilatation to be greater during quinapril administration, but this was not statistically significant (two-way ANOVA: p = 0.12 for quinapril vs. placebo, p < 0.001 for forearm blood flow response, p = ns for interaction).

Plasma fibrinolytic parameters. Substance P and bradykinin caused dose-dependent increases in plasma t-PA antigen and activity concentrations in the infused arm during each study visit (one-way ANOVA: p < 0.001 for all) (Table 1). There was a modest but significant increase in plasma t-PA concentrations in the noninfused arm during infusion of bradykinin, but not substance P. Despite substantial increases in blood flow, sodium nitroprusside had no effect on plasma t-PA concentrations in either arm. During quinapril administration, there was a significant increase in bradykinin, but not substance P, induced increases of plasma t-PA antigen (two-way ANOVA: p = 0.05 for treatment group, p < 0.001 for t-PA response, p = ns for interaction) and activity (p < 0.001 for treatment group, p < 0.001 for t-PA response, p = ns for interaction) concentrations in the infused forearm (Table 1).

There were no significant differences in basal plasma PAI-1 antigen concentrations during placebo, quinapril or losartan administration, or stimulated release of PAI-1 during substance P, sodium nitroprusside or bradykinin infusion (data on file).

Release of t-PA. Substance P and bradykinin caused dose-dependent increases in the plasma t-PA antigen and activity concentration differences between the forearms, and the estimated net release of t-PA antigen and activity during each study visit (one-way ANOVA: p < 0.001 for all).
Table 1. Effect of Placebo, Quinapril and Losartan on Plasma t-PA Antigen and Activity Concentrations in the Infused and Noninfused Forearms During Substance P, Sodium Nitroprusside and Bradykinin Infusion

<table>
<thead>
<tr>
<th>Substance P (pmol/min)</th>
<th>Sodium Nitroprusside (µg/min)</th>
<th>Bradykinin (pmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infused arm</td>
<td>Noninfused arm</td>
<td>Infused arm</td>
</tr>
<tr>
<td>Placebo</td>
<td>Placebo</td>
<td>Placebo</td>
</tr>
<tr>
<td>Plasma t-PA antigen (ng/ml)</td>
<td>Plasma t-PA activity (IU/ml)</td>
<td>Plasma t-PA antigen (ng/ml)</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Infused arm</td>
<td>Noninfused arm</td>
<td>Infused arm</td>
</tr>
<tr>
<td>Placebo</td>
<td>Placebo</td>
<td>Placebo</td>
</tr>
<tr>
<td>Quinapril</td>
<td>Quinapril</td>
<td>Quinapril</td>
</tr>
<tr>
<td>Plasma t-PA antigen (ng/ml)</td>
<td>Plasma t-PA activity (IU/ml)</td>
<td>Plasma t-PA antigen (ng/ml)</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Infused arm</td>
<td>Noninfused arm</td>
<td>Infused arm</td>
</tr>
<tr>
<td>Losartan</td>
<td>Losartan</td>
<td>Losartan</td>
</tr>
<tr>
<td>Plasma t-PA antigen (ng/ml)</td>
<td>Plasma t-PA activity (IU/ml)</td>
<td>Plasma t-PA antigen (ng/ml)</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

*p < 0.001; †p < 0.05; ‡p < 0.001; §p = 0.05. One-way analysis of variance dose response; Two-way analysis of variance: quinapril versus losartan and placebo (p < 0.001 for t-PA response and p = ns for interaction).

t-PA = tissue plasminogen activator.
During quinapril administration, there was a significant increase in bradykinin- but not substance P-induced release of active t-PA (two-way ANOVA: \( p < 0.003 \) for treatment group, \( p < 0.001 \) for t-PA response, \( p = \text{ns} \) for interaction) (Fig. 2) and a trend for t-PA antigen (two-way ANOVA: \( p = 0.09 \) for treatment group, \( p < 0.001 \) for t-PA response, \( p = \text{ns} \) for interaction) (Fig. 2). Quinapril increased the area under the curve for the net release of active t-PA by 135% and 125% in comparison to placebo and losartan respectively.

**DISCUSSION**

For the first time, we have shown that in contrast to AT\(_1\) receptor antagonism, ACE inhibition potentiates bradykinin-induced endogenous t-PA release from the endothelium. However, this potentiation appears to be specific to bradykinin because ACE inhibition did not appear to influence substance-P-induced t-PA release. These findings suggest that the beneficial clinical and vascular effects of ACE inhibition (16) may, in part, be mediated through the acute local augmentation of bradykinin-induced t-PA release.

**Endogenous fibrinolysis and acute coronary syndromes.** The endogenous fibrinolytic system can have important clinical effects, as exemplified by the observation that in a third of patients with an acute myocardial infarction, the infarct-related artery spontaneously reperfuses within 12 h (21–23). Moreover, a low fibrinolytic activity is associated with an increased risk of myocardial infarction in young
men (24) and predicts which patients with unstable angina will develop myocardial infarction (25). Clinical studies of patients with unstable angina have also indicated that there is enhanced activation of the kallikrein system and that bradykinin release is increased (26). Given this augmentation of bradykinin generation and the activation of the intrinsic coagulation pathway in acute coronary syndromes, ACE inhibition may have major beneficial effects on the acute local fibrinolytic balance by markedly enhancing bradykinin-induced t-PA release in areas of intravascular thrombus formation.

**ACE inhibition, bradykinin metabolism and t-PA release.** More than 95% of bradykinin metabolism occurs through ACE, whereas plasma substance P is metabolized by several additional enzymes including dipeptidyl(aminopeptidase IV and aminopeptidase M (27). Moreover, the tissue and cellular metabolism of substance P is performed almost exclusively by neutral endopeptidase 24.11 (27). Consistent with this, and with previous work in the forearm circulation (28), we did not detect a significant influence of ACE inhibition on substance-P-induced vasodilatation. Indeed, ACE inhibition did not appear to influence substance-P-induced t-PA release. This indicates that the augmentation of bradykinin-induced t-PA release reflects the inhibition of bradykinin metabolism rather than a general enhancement of the capacity of the endothelium to release t-PA acutely. Given the recent report (29) of even greater reductions in cardiovascular events with omapatrilat, a combined ACE and neutral endopeptidase inhibitor, the potentiation of bradykinin-induced t-PA release may be enhanced even further by such compounds. This requires further investigation.

In contrast to the marked potentiation of t-PA release, we failed to detect a significant increase in bradykinin-induced vasodilatation during ACE inhibition. Benjamin et al. (15) have previously reported that local ACE inhibition causes a potentiation of the increases in blood flow associated with bradykinin infusion in the human forearm. However, there did appear to be a trend toward an enhanced blood flow response, and it is likely that our study lacked sufficient power to detect this difference. This would also suggest that, in comparison to vasomotor responses, ACE inhibition has a proportionately greater effect on the enhancement of bradykinin-induced t-PA release given more than a doubling of the release of active t-PA. In addition, it is unlikely that the augmentation of active t-PA release by ACE inhibition is due to the potentially greater increase in blood flow because vasodilatation and increased blood flow do not appear, by themselves, to cause t-PA release, as demonstrated by the absence of an effect with sodium nitroprusside infusion. This latter observation is in agreement with our previous studies (5) and work by other groups (4,6).

**Conclusions.** We have demonstrated a specific augmentation of bradykinin-induced t-PA release by ACE inhibition. This effect appears to be independent of angiotensin II action because AT₁ receptor antagonism did not influence the acute release of t-PA. These findings suggest that the beneficial clinical and vascular effects of ACE inhibition may, in part, be mediated through the acute local augmentation of bradykinin-induced t-PA release.

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