Marked Bradykinin-Induced Tissue Plasminogen Activator Release in Patients With Heart Failure Maintained on Long-Term Angiotensin-Converting Enzyme Inhibitor Therapy

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OBJECTIVES The aim of the present study was to assess the contribution of angiotensin-converting enzyme (ACE) inhibitor therapy to bradykinin-induced tissue-type plasminogen activator (t-PA) release in patients with heart failure (HF) secondary to ischemic heart disease.

BACKGROUND Bradykinin is a potent endothelial cell stimulant that causes vasodilatation and t-PA release. In large-scale clinical trials, ACE inhibitor therapy prevents ischemic events.

METHODS Nine patients with symptomatic HF were evaluated on two occasions: during and following seven-day withdrawal of long-term ACE inhibitor therapy. Forearm blood flow was measured using bilateral venous occlusion plethysmography. Intrabrachial bradykinin (30 to 300 pmol/min), substance P (2 to 8 pmol/min), and sodium nitroprusside (1 to 4 pmol/min) were infused, and venous blood samples were withdrawn from both forearms for estimation of fibrinolytic variables.

RESULTS On both study days, bradykinin and substance P caused dose-dependent vasodilatation and release of t-PA from the infused forearm (p ≤ 0.05 by analysis of variance [ANOVA]). Long-term ACE inhibitor therapy caused an increase in forearm vasodilatation (p ≤ 0.05 by ANOVA) and t-PA release (p < 0.001 by ANOVA) during bradykinin, but not substance P, infusion. Maximal local plasma t-PA activity concentrations approached 100 IU/ml, and maximal forearm protein release was ~4.5 µg/min.

CONCLUSIONS Long-term ACE inhibitor therapy augments bradykinin-induced peripheral vasodilatation and local t-PA release in patients with HF due to ischemic heart disease. Local plasma t-PA activity concentrations approached those seen during systemic thrombolytic therapy for acute myocardial infarction. This may contribute to the primary mechanism of the anti-ischemic effects associated with long-term ACE inhibitor therapy. (J Am Coll Cardiol 2002;40:961–6) © 2002 by the American College of Cardiology Foundation

Bradykinin is a potent endothelium-dependent vasodilator that has a brief duration of action due to its rapid degradation by angiotensin-converting enzyme (ACE). In addition to acting as an inflammatory mediator, bradykinin is closely involved in fibrinolytic and coagulation cascades. During the contact phase of blood coagulation, it is released after cleavage of high-molecular-weight kininogen by kallikrein (1). It is also a potent stimulant for the release of tissue-type plasminogen activator (t-PA) from the endothelium (2). Thus, when plaque rupture or erosion activates the intrinsic coagulation pathway, liberation of bradykinin may represent an important negative feedback loop in which bradykinin-induced t-PA release inhibits intravascular thrombus formation.

Large-scale clinical trials of patients with heart failure (HF) or ischemic heart disease indicate a reduction in recurrent infarction rates with ACE inhibitor therapy (3). The mechanisms underlying this anti-ischemic benefit may relate, in part, to the effects on endogenous fibrinolysis. Inhibition of ACE enhances bradykinin-induced vasodilatation and endothelial t-PA release in healthy volunteers (2). However, to date, there has been no assessment of the effect of long-term ACE inhibition on acute endogenous t-PA release in patients with HF or ischemic heart disease. Therefore, the aim of this study was to determine whether long-term ACE inhibition potentiates acute t-PA release in patients with HF secondary to ischemic heart disease.

METHODS

Patients. Nine patients with New York Heart Association functional class II or III HF secondary to ischemic heart...
disease participated in the study, which was undertaken with the approval of the local Research Ethics Committee, in accordance with the Declaration of Helsinki, and each subject gave written, informed consent. All subjects had been maintained on a maximally tolerated dose of an ACE inhibitor for more than six months, and they abstained from alcohol for 24 h and from food and caffeine-containing drinks for at least 4 h before each study.

**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 (2,4). Blood pressure and heart rate were monitored in the noninfused arm at intervals throughout each study by using a semi-automated noninvasive oscillometric sphygmomanometer (Takeda UA 751, Tokyo, Japan).

**ASSAYS.** Venous cannulae (17-gauge) were inserted into large subcutaneous veins of the antecubital fossa in both arms. Ten to 20 ml of blood was withdrawn simultaneously from each arm and collected into acidified buffered citrate (Biopool Stabilyte, Umeå, Sweden; for t-PA assays) and citrate (Monovette, Sarstedt, Nümbrecht, Germany; for plasminogen activator inhibitor type 1 [PAI-1] assays) tubes and kept on ice before being centrifuged at 2,000 g for 30 min at 4°C. Platelet-free plasma was decanted and stored at −80°C before assay. Plasma t-PA and PAI-1 antigen and activity concentrations were determined using enzyme-linked immunosorbent assays and a photometric method, as previously described (2,4).

**Study design.** Patients were evaluated at 9 AM on two occasions: during and after seven-day withdrawal of long-term ACE inhibition therapy. On the appropriate study day, oral ACE inhibition therapy was administered at 8 AM. The brachial artery of the nondominant arm was cannulated with a standard 27-gauge steel-wire needle (Cooper’s Needle Works Ltd., Birmingham, UK) under 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) at 2, 4, and 8 pmol/min, bradykinin (Clinalfa AG) at 30, 100, and 300 pmol/min, and sodium nitroprusside (David Bull Laboratories, Warwick, UK) were given at 1, 2, and 4 μg/min for 10 min at each dose in a randomized order (2). Saline was infused for 30 min before the substance P, sodium nitroprusside, and bradykinin infusions.

**Data analysis and statistics.** Forearm blood flow was calculated for individual venous occlusion cuff inflations, as previously described (2,4). 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 infused forearm blood flow) and the concentration difference between the infused and noninfused arms (2,4). Data were examined, where appropriate, by analysis of variance (ANOVA) with repeated measures and the two-tailed paired Student t test, using Microsoft Excel 97. All results are expressed as the mean value ± SEM. Statistical significance was set at the 5% level.

**RESULTS**

Patient characteristics are shown in Table 1. After withdrawal of ACE inhibition therapy, baseline mean arterial pressure appeared to rise, but this was not statistically significant. There were no significant changes in heart rate, blood pressure or noninfused forearm blood flow (Table 1, Fig. 1) (data on file, University of Edinburgh) during or between the study days.

### Abbreviations and Acronyms

- **ACE** = angiotensin-converting enzyme
- **ANOVA** = analysis of variance
- **d.f.** = degree of freedom
- **HF** = heart failure
- **MI** = myocardial infarction
- **PAI-1** = plasminogen activator inhibitor type 1
- **t-PA** = tissue-type plasminogen activator

### Table 1. Patient characteristics (n = 9)

<table>
<thead>
<tr>
<th>Age yrs (range)</th>
<th>65 (53–79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
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<tr>
<td>Concomitant medications (n)</td>
<td>9</td>
</tr>
<tr>
<td>ACE inhibition</td>
<td>9</td>
</tr>
<tr>
<td>Aspirin</td>
<td>8</td>
</tr>
<tr>
<td>Diuretic</td>
<td>6</td>
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<tr>
<td>Beta-blocker</td>
<td>2</td>
</tr>
<tr>
<td>Statin</td>
<td>8</td>
</tr>
<tr>
<td>Nitrate</td>
<td>4</td>
</tr>
<tr>
<td>Digoxin</td>
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</tr>
<tr>
<td>Calcium antagonist</td>
<td>1</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>1</td>
</tr>
<tr>
<td>Warfarin</td>
<td>3</td>
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<tr>
<td>Echocardiographic parameters</td>
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<tr>
<td>Ejection fraction (%)</td>
<td>25 ± 5</td>
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<tr>
<td>Shortening fraction (%)</td>
<td>10 ± 2</td>
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<tr>
<td>LVEDD (mm)</td>
<td>64 ± 2</td>
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<td>Heart rate (beats/min)</td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>61 ± 5</td>
</tr>
<tr>
<td>Visit 2</td>
<td>61 ± 4</td>
</tr>
<tr>
<td>Mean arterial pressure* (mm Hg)</td>
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</tr>
<tr>
<td>Visit 1</td>
<td>84 ± 4</td>
</tr>
<tr>
<td>Visit 2</td>
<td>89 ± 5</td>
</tr>
<tr>
<td>Forearm blood flow (ml/100 ml/min)</td>
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<td>Visit 1</td>
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<tr>
<td>Noninfused</td>
<td>2.3 ± 0.2</td>
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<tr>
<td>Infused</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Visit 2</td>
<td>Noninfused</td>
</tr>
<tr>
<td>Infused</td>
<td>2.6 ± 0.3</td>
</tr>
</tbody>
</table>

Data are presented as the number of patients or mean value ± SEM. *Diastolic pressure + one-third of pulse pressure.

ACE = angiotensin-converting enzyme; LVEDD = left ventricular end-diastolic diameter; Visit 1 = during long-term ACE inhibition; Visit 2 = after seven-day withdrawal of long-term ACE inhibition.
Forearm blood flow responses. Sodium nitroprusside (data on file), substance P, and bradykinin caused dose-dependent forearm vasodilatation during each study visit (ANOVA for blood flow response, p < 0.001 for all; n = 9 at baseline and with the three doses, degree of freedom (d.f.) = 3) (Fig. 1). In the presence of long-term ACE inhibition, forearm vasodilatation was augmented during bradykinin (ANOVA for ACE inhibition vs. no ACE inhibition, p < 0.05; n = 9 for blood flow at baseline and with the three doses, d.f. = 1), but not substance P or sodium nitroprusside (p = NS) infusion.

Plasma fibrinolytic variables. Bradykinin and substance P caused dose-dependent increases in plasma t-PA antigen and activity concentrations in the infused arm (ANOVA for plasma t-PA concentrations, p < 0.05 for all; n = 9 at baseline and with the three doses, d.f. = 3) (Fig. 1). Plasma t-PA antigen and activity concentrations were significantly augmented during bradykinin (ANOVA for ACE inhibition vs. no ACE inhibition, p < 0.001; n = 9 for plasma t-PA concentrations at baseline and with the three doses, d.f. = 1), but not substance P (p = NS) infusion in the presence of long-term ACE inhibition.

Basal plasma PAI-1 antigen concentrations were lower in the presence (38 ± 6 ng/ml) than in the absence (48 ± 7 ng/ml) of ACE inhibitor therapy (paired t test for ACE inhibition vs. no ACE inhibition, p < 0.02; n = 9 for plasma PAI-1 concentrations, d.f. = 1). Bradykinin and substance P administration had no effect on plasma PAI-1 concentrations (data on file, University of Edinburgh).

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, as well as the estimated net release of t-PA antigen and activity during each study visit (ANOVA for t-PA release, p < 0.01 for all; n = 9 for t-PA release or concentration difference at baseline and with the three doses, d.f. = 3) (Fig. 2). During ACE inhibition, there was a massive increase in bradykinin-induced (ANOVA for ACE inhibition vs. no ACE inhibition, p < 0.001; n = 9 for t-PA release at baseline and with the three doses, d.f. = 1) (Fig. 2), but not substance P–induced (p = NS), release of t-PA antigen and activity (increases in the area under the curve of 520% and 877%, respectively). Post-hoc analysis identified no significant effect of other concomitant medica-

Figure 1. Effect of intra-arterial bradykinin and substance P infusions on blood flow and plasma tissue-type plasminogen activator (t-PA) antigen (solid lines) and activity (dashed lines) concentrations in the infused (solid symbols) and noninfused (open symbols) arms in the presence (left) or absence (right) of angiotensin-converting enzyme (ACE) inhibition. *p < 0.05 by analysis of variance (ANOVA). †p < 0.001 by ANOVA for long-term ACE inhibition versus no ACE inhibition.
DISCUSSION

For the first time, to the best of our knowledge, we have shown that in patients with HF secondary to ischemic heart disease, long-term ACE inhibition markedly potentiates bradykinin-induced endogenous t-PA release from the endothelium. However, this potentiation appears to be specific to bradykinin, because ACE inhibition did not influence substance P–induced t-PA release. These findings suggest that the beneficial clinical and vascular effects of ACE inhibition may be partly mediated through the acute local augmentation of bradykinin-induced t-PA release.

Magnitude of t-PA release. Long-term ACE inhibition produced a massive augmentation of bradykinin-induced t-PA release across the forearm vascular bed. The approximate fivefold increase in t-PA antigen release and the ~20% reduction in plasma PAI-1 antigen concentrations led to the approximate ninefold increase in the release of active t-PA. Our group has previously shown that bradykinin–induced t-PA release is augmented by ACE inhibition in healthy volunteers, but this was modest at approximately twofold only (2). The dramatic potentiation of active t-PA release in the present study is exemplified by the observation that the maximal local forearm concentrations of active t-PA (99 IU/ml at 300 pmol/min of bradykinin) approached those observed during systemic thrombolysis during acute myocardial infarction (MI) (100 to 1,000 IU/ml) (5). Moreover, it also underscores the large capacity of the endothelium to release t-PA quickly—up to 4.5 µg or 16,000 IU/min from the infused forearm at 300 pmol/min of bradykinin. Indeed, using intrabrachial substance P infusions, we have previously demonstrated substantial and sustained release of t-PA for up to 2 h (6).

Minai et al. (7) have recently reported that ACE inhibition produces an approximate twofold increase in bradykinin–induced t-PA release in the coronary circulation of patients with atypical chest pain and angiographically normal coronary arteries. This is consistent with our previous findings in the peripheral circulation of healthy volunteers (2) and suggests that comparable endothelial fibrinolytic effects exist between the peripheral and coronary circulations (8). The present study extends these previous findings, because we have demonstrated a more marked

Figure 2. Estimated net release of tissue-type plasminogen activator (t-PA) antigen (solid lines) and activity (dashed lines) during bradykinin (right) and substance P (left) infusions in the presence (solid circles) or absence (open circles) of angiotensin-converting enzyme (ACE) inhibition. *p < 0.01 by analysis of variance (ANOVA) for all responses. ´p < 0.001 by ANOVA for long-term ACE inhibition versus no ACE inhibition.
augmentation of peripheral t-PA release in patients with HF secondary to ischemic heart disease.

**Mechanism of bradykinin-induced t-PA release.** In keeping with our previous work (2) and that by others (9), ACE inhibition augmented the vasodilatation induced by bradykinin but did not affect the vasodilatation or t-PA release produced by substance P. This suggests that the effect of ACE inhibition does not appear to reflect a generalized enhancement of vascular function but is specific to bradykinin. Brown et al. (10) and Gainer et al. (11) have previously investigated the mechanism of bradykinin-induced t-PA release in the human forearm. Bradykinin induces t-PA release through a B₂ receptor-dependent, nitric oxide synthase-independent, and cyclooxygenase-independent pathway (10). Brown et al. (10) have suggested that bradykinin-induced t-PA release may be caused by an endothelium-derived hyperpolarizing factor, although other mediators may be involved. This group has also described a potential interaction between the vascular responses to bradykinin and the ACE gene insertion/deletion polymorphism (11). We have not explored this interaction because of the small sample size of our study, but this may markedly influence the fibrinolytic response to long-term ACE inhibition in patients with HF or vascular disease, and it requires further investigation.

Inflammation plays an important role in the pathogenesis of HF (12), with elevated plasma concentrations of circulating cytokines such as tumor necrosis factor-alpha (13). Bradykinin receptor expression is altered by ACE inhibition (14,15), inflammation (16), and chronic HF (14,15,17) and may partly explain the proportionately greater and massive release of t-PA from the endothelium.

**Endothelial function, endogenous fibrinolysis, and HF.** The endothelium plays a vital role in the control of blood flow, hemostasis, fibrinolysis, and inflammation. Consequently, the maintenance and regulation of tissue perfusion critically depends on the integrity of endothelial function and the release of potent endothelium-derived factors. After the seminal work of Furchgott and Zawadski (18), it has been widely recognized that an array of mediators can influence vascular tone through endothelium-dependent actions, and there is now extensive evidence of abnormal endothelium-dependent vasomotion that is reversed by ACE inhibition in patients with HF (19,20). However, although endothelium-dependent vasomotion is important, it may not be representative of other aspects of endothelial function, such as the regulation of endogenous fibrinolysis.

Tissue plasminogen activator 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. 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 (21). This dynamic aspect of endothelial function and fibrinolytic balance may be directly relevant to the pathogenesis of atherothrombosis and is not necessarily reflected by the basal plasma concentrations of t-PA (22–24).

**Clinical relevance.** The endogenous fibrinolytic system can have important clinical effects, as exemplified by the observation that in one-third of patients with an acute MI, the infarct-related artery spontaneously reperfu ses within 12 h (25–27). Moreover, low fibrinolytic activity is associated with an increased risk of MI in young men (28) and predicts which patients with unstable angina will develop MI (29). Clinical studies of patients with unstable angina have also indicated that there is an enhanced activation of the kallikrein system and that bradykinin release is increased (30). Given this augmentation of bradykinin generation and 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. This is consistent with the observation that ACE inhibition improves the basal fibrinolytic balance (31,32) and reduces myocardial troponin release in patients with acute coronary syndromes (33).

**Conclusions.** Long-term ACE inhibitor therapy augments bradykinin-induced peripheral vasodilatation and local t-PA release in patients with HF due to ischemic heart disease. Local plasma t-PA activity concentrations approached those seen during systemic thrombolytic therapy for acute MI. This may contribute to the primary mechanism of the anti-ischemic effects associated with long-term ACE inhibitor therapy.

**REFERENCES**