Impaired Capacity for Stimulated Fibrinolysis in Primary Hypertension Is Restored by Antihypertensive Therapy

Wilhelm Ridderstråle, Erik Ulfhammer, Sverker Jern, Thórdís Hrafnkelsdóttir

Abstract—The increased risk for myocardial infarction and ischemic stroke in primary hypertension suggests that the condition is associated with prothrombotic mechanisms. We have shown that patients with hypertension have an impaired capacity for acute endothelial tissue-type plasminogen activator (t-PA) release, an important local protective response to prevent formation of intravascular thrombi. The aim of the present study was to investigate whether this impairment could be restored by the lowering of blood pressure. The capacity for acute t-PA release in response to intraarterial infusion of substance P at 8 pmol/min was investigated in a perfused-forearm study in 20 hypertensive patients (12 men and 8 women). Studies were performed when patients were untreated and after 8 weeks of randomized treatment with lisinopril or felodipine that lowered blood pressure by 26/10 and 24/12 mm Hg, respectively. The t-PA release response increased significantly with treatment (ANOVA, P=0.0001), with a similar effect in the 2 treatment groups. The peak release of t-PA increased from 257 (58) to 445 (77) ng/min×L/tissue⁻¹ (t test, P=0.02). Also, treatment shortened the average time to peak secretion from 6.7 (1.4) to 2.7 (0.3) min (t test, P=0.01). In 6 patients with a delayed secretory peak (9 minutes or later), treatment normalized the response (χ² test, P=0.008). Antihypertensive therapy restores the capacity for acute t-PA release and improves the rapidity of the response in patients with primary hypertension. Similar responses with the 2 regimens suggest that the improvement is related to the blood pressure reduction as such. This effect may contribute to the thromboprotective effect of antihypertensive treatment. (Hypertension. 2006;47:686-691.)

Key Words: endothelium ■ antihypertensive agents ■ calcium antagonists ■ angiotensin antagonists

Primary hypertension increases the risk of cardiovascular events, such as myocardial infarction and ischemic stroke, in a dose-dependent manner.1 The fact that the main complications of hypertension are thrombotic rather than hemorrhagic is somewhat paradoxical, because the primary hemodynamic disturbance in hypertension is exposure of the blood vessels to increased pressure load. Interestingly, increasing clinical and laboratory evidence indicate that hypertension confers a prothrombotic state, although the underlying mechanism of this association is not known.2 In a previous study, we found that the capacity for activation of the endogenous fibrinolytic system by acute release of tissue-type plasminogen activator (t-PA) is markedly impaired in patients with hypertension.3,4 In additional experimental studies, we provided evidence that high intraluminal pressure suppresses the production of t-PA in the vascular endothelium.5 The capacity for a rapid, acute t-PA release from the endothelium is an important local thromboprotective mechanism to prevent formation of occluding thrombi when intravascular clot formation is initiated.6 In vivo, the fibrinolytic response is evoked by products released by activated platelets and coagulation factors. If the capacity for acute t-PA release is defective, as we found it to be in hypertensive patients, the likelihood of timely spontaneous thrombolysis in case of, for instance, a plaque rupture, may be reduced.

Pharmacological treatment of hypertension reduces the incidence of atherothrombotic events, and its beneficial effects have been suggested to be greater than what would be expected to result from a slowing of the atherosclerotic process alone.7 In view of our previous observations, restoration of the capacity for a powerful local fibrinolytic response by antihypertensive therapy could be one such effect. The primary aim of the present study was to investigate whether lowering of blood pressure can restore t-PA synthesis and thereby improve the capacity for acute t-PA release in hypertensive patients. To differentiate drug-specific effects from those of the blood pressure reduction, as such, we used 2 drugs with different modes of action (the angiotensin-converting enzyme inhibitor and calcium antagonist lisinopril and felodipine, respectively), aiming at a similar blood pressure reduction with both agents.
Methods

Subjects
We studied 20 white subjects (age, 61 years; range, 39 to 75 years; 12 men and 8 women) with documented primary hypertension. All of the women were postmenopausal. Baseline characteristics are shown in Table 1. All of the subjects were nonsmokers without a history of diabetes mellitus or other major illness and were on no other medication than antihypertensive drugs. The patients were recruited by advertisement, and secondary hypertension was excluded by standard procedures. Patients with blood lipid derangements or impaired glucose tolerance were not included.

After study day 1, patients were randomized to open treatment with either lisinopril or felodipine. The patients in the lisinopril group had, on the average, a longer duration of hypertension of 15 years compared with 5 years in the felodipine group. The severity of hypertension was similar in the 2 groups, with 3 and 2 patients, respectively, on dual therapy on enrollment in the felodipine and lisinopril group. Three subjects in the felodipine group and 1 subject in the lisinopril group were previously untreated. The remaining patients were on monotherapy. In the felodipine group, 3 patients had been treated previously by angiotensin-converting enzyme (ACE) inhibitors and 2 patients by calcium channel blockers. In the lisinopril group, 5 patients previously had an ACE inhibitor, and 2 had a calcium channel blocker. A smaller number of subjects in both groups were treated with β blockers, angiotensin II receptor blockers, and diuretics.

The study protocol was approved by the Ethics Committee of the Göteborg University and conducted according to the Declaration of Helsinki. The nature, purpose, and potential risks of the study were carefully explained to each subject before informed consent was obtained.

Study Design
Studies began ~4 weeks after cessation of antihypertensive treatment, and patients with blood pressure levels >140 mm Hg systolic and 90 mm Hg diastolic were included. After a baseline examination including 24-hour blood pressure monitoring, patients went through an invasive perfused-forearm study to determine the capacity for stimulated t-PA release and endothelium-dependent vasodilation. Thereafter, patients were randomized to open treatment with either lisinopril (Zestril) at 10 mg or felodipine (Plendil) at 5 mg daily. The dosage was individually titrated to approach target levels of blood pressure (130/85 mm Hg) or, if this was not achieved, to a maximal dose of lisinopril at 20 mg or felodipine at 15 mg daily. After ~8 weeks of treatment on target levels or with maximal drug dose, a second perfused-forearm study was performed according to an identical protocol as the first examination.

Perfused-Forearm Study
The experimental procedure has been described previously in detail.9,10 In brief, the subject attended the laboratory after an overnight fast. On the second experimental day, the patients took their study medication in the morning. An 18-gauge arterial polyethylene catheter was introduced percutaneously with modified Seldinger technique into the brachial artery of the nondominant arm. An indwelling cannula was placed retrogradely into a deep ipsilateral antecubital vein. Intraarterial blood pressure was recorded by an electrical transducer connected to a SC 9000 monitor (Siemens Medical Systems Inc). After each venous blood sampling, forearm blood flow (FBF) was assessed by venous occlusion plethysmography with a mercury-in-silastic strain gauge using the MAPPC software (Elektromedicin AB). Means of 3 to 5 recordings were expressed in milliliters per minute and liters of tissue. Intraobserver and interobserver coefficients of variation were, on the average, 5.6% and 4.6%, respectively.

After cannulation of the artery, ~45 minutes were allowed before baseline recordings (15 minutes). Thereafter, substance P at 8 pmol/mL (substance P; CLINALFA) was infused for 20 minutes into the brachial artery at a constant rate of 1 mL/min. Postinfusion recordings were performed for 20 minutes. The dose of substance P was chosen to obtain maximal t-PA release response without concomitant systemic effects.11

During preinfusion and postinfusion baseline periods, blood samples were collected simultaneously from the brachial artery and vein. During substance P infusion, venous blood samples were obtained at 1.5, 3, 6, 9, 12, 15, and 18 minutes. To avoid interruption of the infusion, arterial blood was obtained only at baseline and the end of the infusion and in-between values interpolated from these values. Blood was collected in chilled tubes containing 0.1 vol of 0.45 mol/L sodium citrate buffer (pH 4.3; Stabilyte, Biopool AB) for determination of fibrinolytic proteins. Tubes were kept on ice until plasma was isolated by centrifugation at 4°C and 2000g for 20 minutes. Plasma aliquots were immediately frozen and stored at −70°C until assay.

Biochemical Analyses
Plasma concentrations of total t-PA and plasminogen activator inhibitor (PAI) 1 were determined by ELISA (TintElize t-PA, Biopool International; Coalize PAI-1, Chromogenix AB). Both assays detect free and complexed forms of the respective proteins with equal efficiency.12,13 Active t-PA and PAI-1 were analyzed by a biofunctional immunosorbant assays (Chromolize t-PA and Chromolize PAI-1, Biopool International). In our laboratory, the lowest detectable levels are 1.0 and 1.4 ng/mL for t-PA and PAI-1 antigen and 0.03 and 2 IU/mL for t-PA and PAI-1 activity, respectively (data on file). Samples from both experiments were assayed in duplicate on the same microtest plate. Total t-PA was analyzed at all points of blood collection, whereas t-PA activity was measured during the peak release, that is, the first 6 minutes of infusion. PAI-1 antigen and activity was only measured at baseline, because previous studies indicate that there is no releasable pool of PAI-1 in the endothelium.14,15 Intraassay variation coefficients were <5%. High-sensitive C-reactive protein was analyzed by chemiluminescent immunometric assay (IMMULITE 2000). Blood chemistry analyses were performed by standard methods at the Department of Clinical Chemistry at the Sahlgrenska University Hospital.

Calculations
Forearm vascular resistance (FVR) was calculated as the ratio of mean arterial pressure to FBF and expressed in arbitrary resistance units. FBF was interconverted to forearm plasma flow by hematocrit. Venoarterial concentration gradients were obtained by subtraction of readings in simultaneously collected venous and arterial samples. Net release or uptake rates for fibrinolytic proteins were calculated as the venoarterial concentration gradient times forearm plasma flow. Total cumulative t-PA release in response to substance P was estimated for each individual as area under the curve from baseline until 20 minutes after terminating the infusion. In this analysis, negative areas (ie, net uptake) were ignored.

Statistical Analysis
Standard statistical methods were used. Unless otherwise stated, values are presented as mean and SEM. Between-treatment status
comparisons of single variables were performed by Student t test. Responses to substance P were evaluated by 2-way (treatment/no treatment and time) and 1-way (time) ANOVA for repeated measures. Between-drug comparisons of responses were performed by 2-way ANOVA during treatment (drug and time). Proportions of categorical data were compared by χ² test. Findings were considered significant at P<0.05 (2-tailed tests).

Results

Baseline Hemodynamic and Fibrinolytic Variables

Baseline hemodynamic and fibrinolytic variables are shown in Table 2. After target blood pressure levels were reached, the patients were treated on average for 10 and 9 weeks in the lisinopril and felodipine groups, respectively, before the second study day was performed. Treatment lowered the intraarterial systolic and diastolic blood pressure on the average from 165(3)/82(2) to 140(3)/71(1) mm Hg (P<0.01 throughout). Changes in blood pressure were similar in the lisinopril and felodipine groups, or 24/12 and 26/10 mm Hg, respectively (P value was not significant). Baseline FBF and FVR were not affected by treatment. Also, baseline concentrations of the fibrinolytic variables did not change significantly in either group by treatment. Thus, t-PA activity was 0.64 (0.05) and 0.68 (0.07) IU/mL in untreated and 0.66 (0.05) and 0.68 (0.09) IU/mL in patients treated with felodipine and lisinopril (P value was not significant for both), respectively. Also, PAI-1 activity was 6.1 (1.1) and 6.7 (2.5) IU/mL in untreated and 0.66 (0.05) and 0.68 (0.05) IU/mL in patients treated with felodipine and lisinopril (P value was not significant for both), respectively.

TABLE 2. Summary of Baseline Hemodynamic, Inflammatoric, and Fibrinolytic Variables

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated</th>
<th>Treated</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure,* mm Hg</td>
<td>165.4 (3.0)</td>
<td>140.3 (3.4)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Diastolic blood pressure,* mm Hg</td>
<td>81.9 (1.5)</td>
<td>71.1 (1.4)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Mean arterial pressure,* mm Hg</td>
<td>115.3 (1.7)</td>
<td>98.7 (1.9)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Forearm blood flow, mL/L tissue</td>
<td>62.3 (7.2)</td>
<td>47.7 (4.8)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Forearm vascular resistance, arbitrary units</td>
<td>2.5 (0.3)</td>
<td>2.6 (0.3)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Plasma t-PA antigen, ng/mL</td>
<td>8.9 (0.5)</td>
<td>8.6 (0.6)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Plasma t-PA activity, IU/mL</td>
<td>0.66 (0.05)</td>
<td>0.68 (0.05)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Plasma PAI-1 antigen, ng/mL</td>
<td>31.1 (3.2)</td>
<td>29.8 (6.2)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Plasma PAI-1 activity, IU/mL</td>
<td>6.1 (1.1)</td>
<td>6.7 (2.5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Hs-CRP, mg/L</td>
<td>3.6 (0.4)</td>
<td>2.7 (0.5)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Data are mean and (SEM). n.s. indicates not significant; Hs-CRP, high-sensitive C-reactive protein.

*Blood pressure was measured intraarterially at the beginning of the experiment.

Figure 1. Net forearm release rates of t-PA antigen during baseline and in response to 20 minutes of intraarterial infusion of substance P (8 pmol/min) in untreated (○) and treated (●) hypertensive patients (baseline measurements 15 minutes before and 20 minutes after the infusion), 2-way ANOVA, mean and SEM.

Figure 2. Net forearm release rates of t-PA antigen during baseline and in response to 20 minutes of intraarterial infusion of substance P (8 pmol/min) in patients on felodipine (○) or lisinopril (●) treatment (baseline measurements 15 minutes before and 20 minutes after the infusion). P refers to 2-way ANOVA (drug x time), mean and SEM.

Substance P induced highly significant t-PA secretory responses of the forearm, both when patients were untreated and when they were on active antihypertensive treatment (ANOVA, P<0.0001). In line with the hypothesis, the t-PA antigen release response was significantly greater on treatment (Figure 1; 2-way ANOVA, P=0.0001). There were no significant differences in the t-PA release responses between the treatment groups (Figure 2), although the increase in t-PA release was somewhat larger in the lisinopril-treated group (P value was not significant). The cumulated t-PA antigen release during substance P infusion increased from 3000 (655) to 4557 (701) ng/L tissue with treatment (t test, P<0.05). The release of active t-PA during the first 6 minutes of infusion was significantly improved by treatment (2-way ANOVA, P=0.03).

The t-PA antigen release, which was in the order of 9.5 and 11.8 ng/min and L tissue at baseline, increased significantly and peaked at 257 (58) and 445 (77) ng/min and L tissue during the substance P infusion, in untreated and treated patients, respectively (P<0.0001 for both). The peak t-PA release was significantly improved by treatment (t test, P=0.02) and was of almost identical magnitude in the lisinopril and felodipine groups (P value was not significant). On the whole, substance P induced a 27- and 38-fold increase in t-PA release.
release in untreated and treated patients, respectively (P < 0.05 for change in fold increase).

Antihypertensive treatment also altered the temporal response pattern to stimulation (Figure 3). When patients were untreated, one-third of the patients had a delayed onset of the t-PA response; in 6 of 20 patients, the peak release rate occurred 9 minutes or later after initiating substance P stimulation. The response pattern was normalized with treatment, and on the second study day, all of the patients had the peak release rate during the first 6 minutes of stimulation (χ² test, P = 0.008). Thus, treatment improved the response pattern and shortened the average time to peak secretion from 6.7 (1.4) to 2.7 (0.3) min (t test, P = 0.01). Again, the improvement of the temporal response pattern was similar in the 2 treatment groups.

Vasodilator Responses
Substance P induced highly significant decreases in FVR and increases in FBF, both when patients were untreated and on active treatment (ANOVA, P < 0.0001 for all). The responses of FVR (Figure 4) and FBF to substance P stimulation were of the same magnitude on both treatment days (2-way ANOVA, P value was not significant).

Discussion
The findings of the present study show for the first time that the capacity for stimulated t-PA release can be significantly improved by blood pressure lowering. Not only did treatment increase the amount of t-PA released, but it also improved the temporal pattern of the response with an earlier onset and a higher peak release rate. In all 6 of the patients with a delayed t-PA release response, the time to peak was normalized. The changes were of similar magnitude in the lisinopril and felodipine groups, suggesting that the improvement was related to the blood pressure effect per se.

Acute release of t-PA, which, in vivo, is induced by activation of platelets and the coagulation cascade, is an important protective mechanism to prevent formation of occlusive intravascular thrombi.14 The importance of t-PA-mediated plasminogen activation for maintenance of vascular patency has been confirmed in gene targeting and gene transfer studies.6 In keeping with the hypothesis, we showed recently that subjects with a genotype associated with a low capacity for t-PA secretion had an increased risk of myocardial infarction in a population-based, prospective epidemiological study.15 Also, a similar association has been found between the low-secretor enhancer genotype and risk of lacunar stroke.16

Our previous in vivo studies in humans and in experimental porcine models have shown that the acute t-PA response is rapid and powerful under physiological conditions.5,17–19 Indeed, a prompt onset of the response with a short delay until the peak of the secretory response is probably pivotal for its antithrombotic effect, because t-PA is ≈2 orders of magnitude more effective when t-PA is present during clot formation than in dissolving an existing thrombus.20,21 In healthy humans, acute release of t-PA can be initiated within <1 minute, and the release rate can increase manifold with stimulation.9,19,22,23 If the response is slow and defective, as it appears to be in patients with untreated hypertension, it may reduce the potential for a timely activation of the fibrinolysis in case of an atherothrombotic event.

Several observations suggest that lowering blood pressure may reduce the risk of atherothrombosis.5 Restoration of the capacity for an effective fibrinolytic response could be one of the looked-for mechanisms behind this effect. Despite its potential implications, however, the effects of antihypertensive therapy on the capacity for activation of the local fibrinolytic system in hypertension have not been defined. A few previous studies have investigated the effect of blood pressure–lowering drugs on the plasma levels of fibrinolytic factors. In contrast to a calcium antagonist and an angiotensin II type 1 receptor blocker, 2 ACE inhibitors (enalapril and quinapril) improved plasma fibrinolysis.24,25 These findings suggest that ACE inhibition has a more favorable effect on t-PA production. Unfortunately, local endothelial t-PA secretion rates cannot be inferred from these studies, because of the following: (1) mixed venous plasma t-PA represents the integrated release from the whole vasculature, and (2) the rapid degradation of t-PA by the liver makes steady-state levels very sensitive even to minute changes in hepatic clearance.26 Furthermore, as shown by us in a previous study,27 baseline plasma levels do not predict the individual’s capacity for activation of the acute release response, which is likely to be the key determinant of a successful clot resolution.
More specifically investigating the effect of ACE inhibition on the capacity for stimulated local t-PA release, Minai et al.23 and Matsumoto et al.28 studied the response to intra-coronary infusion of bradykinin in patients with atypical chest pain and hypertension, respectively. In both studies, ACE inhibition was found to potentiate bradykinin-induced t-PA release, and a similar effect was also reported in a forearm study.29,30 However, these observations do not necessarily indicate that there is a specific improvement in endothelial t-PA synthesis or release capacity, because it cannot be ruled out that the effect is because of an increased bioavailability of bradykinin when its degradation is blocked by inhibition of ACE. Indeed, a recent study by Pretorius et al.31 confirmed that the effect of ACE inhibition on t-PA release was bradykinin dependent, and the effect could be abolished by the bradykinin B₂-receptor blocker HOE 140. To avoid a confounding effect of this potential pharmacological interaction, we used substance P in the present study to ensure that t-PA release was induced through a bradykinin-independent pathway. Thus, our findings clearly suggest a cellular mechanism to be responsible for the improved t-PA secretory response by antihypertensive therapy in hypertensive patients.

Taken together with our previous observations, it is likely that the impaired capacity for t-PA release in hypertensive patients reflects a diminished protein synthesis with depletion of the intracellular storage pools from which it is released on stimulation.32 The fact that an improved fibrinolytic capacity was observed with both drugs supports the concept that the effect is primarily related to the blood pressure decrease as such. However, the observation that the improvement was somewhat greater with the ACE inhibitor may indicate that there are additional, drug-specific effects. This important issue needs to be addressed in larger studies.

In keeping with the interpretation that the improvement was mainly related to the lowering of blood pressure, we have shown previously in an ex vivo perfusion model that exposure of intact conduit vessels to elevated intraluminal pressure causes a suppression of t-PA gene expression and protein release.5 To additionally characterize the specific force component responsible for this effect, we have demonstrated recently that t-PA expression is downregulated by prolonged (48 to 72 hour) cyclic tensile stimulation of endothelial cells.33 Hence, these findings support the concept that it is the increased tensile stress imposed on the endothelium by the high blood pressure that could be the mechanism behind the reduced endothelial storage pool of t-PA in hypertension.

In contrast to the improvement in t-PA release, we found no effect of blood pressure treatment on the vasodilatory capacity of the endothelium. Thus, the regulation of endothelial fibrinolysis and vasodilation appears, at least to some extent, to be dissociated. Previous studies on the effect of chronic blood pressure treatment have shown either no change34,35 or improvement36–39 in the vasomotor functions. The positive findings have been most consistent for treatment with ACE inhibitors and calcium channel blockers and when bradykinin or acetylcholine/methacholine have been used as stimuli. To our knowledge, the effects of blood pressure lowering on substance P-induced vasodilation have not been described previously.

In conclusion, the impaired capacity for acute release of t-PA from the vascular endothelium in patients with primary hypertension can be restored by antihypertensive therapy. Because a rapid and powerful release of t-PA is essential for prevention of thrombus formation in case of intravascular clot formation, this observation provides a novel mechanism behind the preventive effect of blood pressure-lowering therapy on the risk for atherothrombotic events.

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References


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