Atherosclerosis and thus cardiovascular events.1 In addition to um, secondary to reduced NO availability, acts to promote and release of nitric oxide (NO). A dysfunctional endothelial participates in the activation of endogenous fibrinolysis,3 another the main activator of endogenous fibrinolysis.3,4 Experimental evidence in healthy humans, NO promotes the against the development of atherothrombosis. According to crucial mechanism whereby NO may protect the vessel wall its well-documented effects on vascular function,2 NO par-

**Key Words:** plasminogen activators ■ endothelium ■ nitric oxide ■ endothelium-derived hyperpolarization factor ■ hypertension

Vascular endothelium plays a primary role in the modula-
tion of vascular tone and structure by the production and release of nitric oxide (NO). A dysfunctional endotheli-
um, secondary to reduced NO availability, acts to promote atherosclerosis and thus cardiovascular events.1 In addition to its well-documented effects on vascular function,2 NO participates in the activation of endogenous fibrinolysis,3 another crucial mechanism whereby NO may protect the vessel wall against the development of atherothrombosis. According to experimental evidence in healthy humans, NO promotes the release of tissue-type plasminogen activator (tPA), which is the main activator of endogenous fibrinolysis.5,4

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**Clinical Perspective p 1633**

Essential hypertension is a clinical condition characterized by endothelial dysfunction. A major aspect of this alteration concerns reduced NO availability secondary to oxidative stress, which leads to both reduced endothelium-dependent vasodilation5–7 and impaired capacity of tPA release.4 Endothelial cells produce other relaxing factors, including endothelium-derived hyperpolarizing factor (EDHF), that cause hyperpolarization of smooth muscle cells.8 In several experimental models and clinical conditions, such as essential hypertension, EDHF induces vasodilation as a rapid compensatory mechanism for decreased NO availability.9–11 Production of EDHF involves the activation of cytochrome P450 epoxygenase (CYP 2C9), which is expressed mainly within endothelial cells.9,12,13 CYP 2C9 generates metabolites of arachidonic acid epoxidecatrienoic acids (EETs), which either initiate endothelial cell hyperpolarization or are released from endothelial cells to stimulate potassium in vascular smooth cells.10,12 It has been reported recently that

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1625
EETs also possess fibrinolytic properties via modulation of tPA release. Additional experimental findings suggest that physiological concentrations of EETs increase tPA expression in endothelial cells, whereas EETs contribute to tPA release from human umbilical vein endothelial cells, an effect inhibited by miconazole, a selective CYP 2C9 inhibitor. However, to date, a possible modulating effect of EDHF on endogenous fibrinolysis has not been evaluated in humans. In humans, in vivo, the vascular activity of CYP 2C9 might be blocked by sulfaphenazole, a compound that selectively blocks this pathway in vitro. Therefore, the first aim of the present study was to investigate the possible role of CYP 2C9–derived EDHF in the modulation of tPA release in forearm microcirculation of normotensive subjects. In addition, because reduced NO availability and a compensatory vasodilation response to EDHF production are documented in essential hypertension, the possible role of CYP 2C9 in modulating endothelial tPA release in patients with essential hypertension was also assessed.

Methods

Subjects

The study population included 56 healthy male volunteers and 57 male patients with essential hypertension. The 2 groups were matched for age by the group-matching method, and the age range used was from 30 to 60 years. Patients were recruited from newly diagnosed cases in the outpatient clinic. The inclusion criterion was seated blood pressure values (after 10 minutes of rest) between 140/90 and 160/99 mm Hg, confirmed on 2 separate occasions within 1 month according to European guidelines. Exclusion criteria were dyslipidemia, diabetes mellitus, smoking, body mass index >30 kg/m², renal or liver impairment, and established cardiovascular disease other than essential hypertension. In addition, female gender was considered an exclusion criterion for 2 main methodological reasons. The first was to avoid the confounding effect of menopause, given that patients with an age range of 30 to 60 years were included in the study. The second issue concerned the higher failure of cannulation of a deep forearm vein in women, with the risk of selecting a population characterized by a nonhomogeneous gender distribution.

Secondary forms of hypertension were excluded by routine diagnostic procedures. Patients either were never treated for hypertension or they had not received any medication for ≥1 month before enrollment in the study. The study protocol was approved by the local ethics committee and performed according to the guidelines of our institution. All patients were aware of the nature, purpose, and potential risks of the study and gave their written informed consent to participate.

Experimental Procedures

The perfused-forearm model used in the present study has been described previously in detail. Briefly, intravenous catheters were placed in deep antecubital veins of each arm (experimental and contralateral forearm), and the brachial artery was cannulated for drug infusion at systemically ineffective rates and for intra-arterial blood pressure and heart rate monitoring. Forearm blood flow (FBF) was measured in both forearms by strain-gauge venous plethysmography (EC-6, D.E. Hokanson Inc, Bellevue, Wash). Before FBF measurement, simultaneous arterial and venous samples were obtained from the infused arm before and after each dose of study drugs. Infusions were interrupted during arterial sampling. Plasma concentrations of tPA antigen were determined by ELISA (Technoclone GmbH, Vienna, Austria). All samples were assayed in duplicate on the same test plate. Details concerning the methods performed in our laboratory, including sensitivity and reproducibility, have been published previously.

Experimental Design

Contribution of CYP 2C9–Derived Hyperpolarizing Factor to Bradykinin-Mediated tPA Release in Normotensive Subjects and Patients With Essential Hypertension

In 22 normotensive subjects (mean age 42±4 years) and 20 hypertensive patients (mean age 48±6 years), tPA release was estimated after an intra-arterial infusion of bradykinin (0.015 μg · 100 mL⁻¹ · min⁻¹), which was infused for 10 minutes. To assess the contribution of CYP 2C9–derived EDHF on endothelial tPA release, the infusion of bradykinin was repeated in the presence of sulfaphenazole (0.03 μg · 100 mL⁻¹ · min⁻¹), a highly selective CYP 2C9 inhibitor. To rule out any possible interference of cyclooxygenase-derived vasoactive prostanoids on tPA release, additional groups of 6 normotensive subjects (mean age 44±5 years) and 6 hypertensive patients (mean age 47±5 years) were given oral acetylsalicylic acid (1 g) 2 hours before the study.

The infusion rate of bradykinin was determined according to preliminary experiments that aimed to test the effect of increasing doses of bradykinin (0.005, 0.015, and 0.05 μg · 100 mL⁻¹ · min⁻¹) on tPA release. The selection of the intermediate dose was based on the balance between a positive effect on tPA release and a vasodilation level that was not too high, because extreme vasodilation could be a confounding factor in the calculation of net balance, which requires a stabilized FBF.

To exclude the possible confounding effect of flow increase, intra-arterial sodium nitroprusside (1.0 μg · 100 mL⁻¹ · min⁻¹), a direct smooth muscle cell relaxant compound, was also infused. After 10 minutes of sulfaphenazole preinfusion, bradykinin was infused for 10 minutes and continued throughout. A 30-minute washout was allowed between each dose-response curve.

Contribution of NO and CYP 2C9–Derived Hyperpolarizing Factor to Acute tPA Release in Normotensive Subjects and Patients With Essential Hypertension

The present series was designed to assess the effect of EDHF on stimulated tPA release in the absence and presence of NO inhibition. Thus, in 21 normotensive subjects (mean age 43±3 years) and 23 hypertensive patients (mean age 48±7 years), bradykinin was administered during infusions of saline (0.2 mL/min), sulfaphenazole (0.03 μg · 100 mL⁻¹ · min⁻¹), or the NO synthase inhibitor N⁶-monomethyl-L-arginine (L-NMA; 100 μg · 100 mL⁻¹ · min⁻¹), as well as during L-NMA and sulfaphenazole coniusions. To further investigate the effect of CYP 2C9 on tPA release, the endothelial agonist acetylcholine (1.5 μg · 100 mL⁻¹ · min⁻¹) was infused in additional groups of normotensive subjects and hypertensive patients (n=8 each group) during saline (0.2 mL/min), sulfaphenazole (0.03 μg · 100 mL⁻¹ · min⁻¹), and/or L-NMA (100 μg · 100 mL⁻¹ · min⁻¹). The dose of acetylcholine was selected on the basis of previous results.

Under L-NMA, infusions were performed according to the NO clamp technique, which enables assessment of endothelial agonists in the presence of NO synthase blockade without a change in basal blood flow, thus avoiding any perturbation that could alter net tPA balance. Briefly, after 10 minutes of L-NMA infusion, sodium nitroprusside was infused at an adjusted dose (0.3 and 0.4 μg · 100 mL⁻¹ · min⁻¹) to neutralize the L-NMA–induced vasodilation and restore baseline FBF, as previously described in detail.

In each series, the sequence of the agonists was randomized. Sulfaphenazole and L-NMA were started 10 minutes before bradykinin and acetylcholine and continued throughout. A 30-minute washout was allowed between each infusion, whereas a 60-minute period was allowed when L-NMA was infused.
Table 1. Clinical Characteristics of Study Group

<table>
<thead>
<tr>
<th></th>
<th>Normotensive Subjects (n=56)</th>
<th>Hypertensive Patients (n=57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>42±6</td>
<td>48±7*</td>
</tr>
<tr>
<td>Smoker, yes/no</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>130±7</td>
<td>153±6*</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>81±5</td>
<td>95±4*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23±4</td>
<td>24±2</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L (mg/dL)</td>
<td>5.31±0.57 (205±22)</td>
<td>5.36±0.57 (207±22)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L (mg/dL)</td>
<td>1.42±0.36 (55±14)</td>
<td>1.37±0.31 (53±12)</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L (mg/dL)</td>
<td>3.08±0.72 (119±28)</td>
<td>3.19±0.82 (123±32)</td>
</tr>
<tr>
<td>Plasma glucose, mmol/L (mg/dL)</td>
<td>4.77±0.33 (86±6)</td>
<td>4.88±0.44 (88±8)</td>
</tr>
<tr>
<td>Plasma homocystine, µmol/L</td>
<td>9.7±2.3</td>
<td>9.8±3.0</td>
</tr>
<tr>
<td>Plasma folate, mmol/L (ng/mL)</td>
<td>18.6±10.7 (8.2±4.7)</td>
<td>18.1±8.8 (8.0±3.9)</td>
</tr>
<tr>
<td>Plasma vitamin B12, pmol/L (pg/mL)</td>
<td>294.1±37.7 (398.5±51.1)</td>
<td>221.4±47.08 (300.1±63.8)</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>3.0±1.1</td>
<td>2.8±1.2</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; and CRP, C-reactive protein. Data are presented as mean±SD. *P<0.05 vs normotensive subjects.

Results

Clinical characteristics of the study population are shown in Table 1. In accordance with exclusion criteria, groups were similar in characteristics except with regard to systolic and diastolic blood pressure values, which were significantly higher in the hypertensive group. Age was slightly but significantly higher in hypertensive patients than in normotensive subjects. During intrabrachial drug infusion, no change in intra-arterial blood pressure or heart rate was observed (data not shown).

Effects of Bradykinin on tPA Release in Normotensive Subjects and Patients With Essential Hypertension

Vasodilation in response to bradykinin was significantly (P<0.05) higher in normotensive subjects than in hypertensive patients (Figure 1). At baseline, normotensive subjects showed higher arterial and venous concentrations of tPA than hypertensive patients (Table 2). In the normotensive group, venous concentrations of tPA increased significantly during bradykinin infusion (Table 2). By contrast, in hypertensive patients, no changes in tPA venous concentrations were detected during bradykinin infusion (Table 2). Because arterial tPA concentrations were not affected by drug infusion in any group, the venous concentration gradient of tPA increased significantly after bradykinin infusion in normotensive subjects but not in hypertensive patients (Table 2). As a consequence, stimulated tPA release was significantly (P<0.05) greater in healthy subjects (Figure 2A) than in hypertensive patients (Figure 2B).
Intrabrachial infusion of sodium nitroprusside, which induced a similar vasodilation in normotensive subjects (FBF from 2.8±0.5 to 12.1±1.8 mL·min⁻¹·100 mL⁻¹ forearm tissue) and in hypertensive patients (FBF from 2.9±0.7 to 12.8±2.1 mL·min⁻¹·100 mL⁻¹ forearm tissue) failed to induce any significant increase in either venous or venous-arterial concentration gradients of tPA (Table 2). Therefore, no significant increase of tPA balance was found in normotensive subjects (from 0.02±0.01 to 0.12±0.08 ng·min⁻¹·100 mL⁻¹ forearm tissue) or in hypertensive patients (from 0.03±0.02 to 0.14±0.10 ng·min⁻¹·100 mL⁻¹ forearm tissue).

Contribution of CYP 2C9–Derived Hyperpolarizing Factor to Bradykinin-Mediated tPA Release in Normotensive Subjects and in Patients With Essential Hypertension

In both the normotensive and hypertensive groups, sulfaphenazole preinfusion did not significantly change basal FBF (normotensive group: from 2.9±0.8 to 3.0±0.8 ng·min⁻¹·100 mL⁻¹ forearm tissue; hypertensive group: from 3.2±1.2 to 3.3±1.4 ng·min⁻¹·100 mL⁻¹ forearm tissue). Sulfaphenazole infusion, which did not affect endothelium-dependent relaxation in normotensive subjects (Figure 1A), significantly blunted the vasodilation response to bradykinin in hypertensive patients (Figure 1B). Moreover, in normotensive subjects, sulfaphenazole, which basally failed to affect tPA release (from 0.10±0.02 to 0.18±0.05 ng·min⁻¹·100 mL⁻¹ forearm tissue), significantly reduced bradykinin-induced tPA release (Figure 2A). A similar response was obtained in hypertensive patients, in whom sulfaphenazole did not significantly change basal tPA release (from 0.13±0.30 to 0.16±0.05 ng·min⁻¹·100 mL⁻¹ forearm tissue), whereas it reduced bradykinin-mediated tPA release (Figure 2B).

Cyclooxygenase inhibition did not significantly affect vasodilation in response to bradykinin either in the absence or the presence of sulfaphenazole in either normotensive subjects or hypertensive patients (data not shown). Similarly, tPA release was unaffected by cyclooxygenase inhibition in both normotensive subjects (bradykinin plus saline: from 0.14±0.02 to 1.8±0.05 ng·min⁻¹·100 mL⁻¹ forearm tissue; bradykinin plus sulfaphenazole: from 0.20±0.04 to 0.82±0.11 ng·min⁻¹·100 mL⁻¹ forearm tissue) and hypertensive patients (bradykinin plus saline: from 0.11±0.03 to 0.56±0.35 ng·min⁻¹·100 mL⁻¹ forearm tissue; bradykinin plus sulfaphenazole: from 0.18±0.02 to 0.22±0.13 ng·min⁻¹·100 mL⁻¹ forearm tissue). Finally, sulfaphenazole did not alter vasodilation and tPA release in response to sodium nitroprusside in either group (data not shown).

Contribution of NO and CYP 2C9–Derived Hyperpolarizing Factor to Acute tPA Release in Normotensive Subjects and Patients With Essential Hypertension

In this group, a greater (P<0.01) vasodilation response to bradykinin and to acetylcholine in normotensive subjects than

<table>
<thead>
<tr>
<th>Drug Dose*</th>
<th>Arterial tPA</th>
<th>Venous tPA</th>
<th>Arteriovenous tPA</th>
<th>Arterial tPA</th>
<th>Venous tPA</th>
<th>Arteriovenous tPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.31±0.35</td>
<td>1.36±0.35</td>
<td>0.05±0.06</td>
<td>0.72±0.12*</td>
<td>0.73±0.12*</td>
<td>0.01±0.03</td>
</tr>
<tr>
<td>Bradykin (0.015)</td>
<td>1.33±0.36</td>
<td>1.46±0.38†</td>
<td>0.13±0.07†</td>
<td>0.73±0.11</td>
<td>0.79±0.13</td>
<td>0.06±0.02†</td>
</tr>
<tr>
<td>Baseline</td>
<td>1.35±0.34</td>
<td>1.36±0.33</td>
<td>0.01±0.04</td>
<td>0.72±0.11*</td>
<td>0.73±0.18*</td>
<td>0.01±0.05</td>
</tr>
<tr>
<td>SNP (1.0)</td>
<td>1.34±0.35</td>
<td>1.35±0.32</td>
<td>0.01±0.06</td>
<td>0.73±0.11</td>
<td>0.74±0.11</td>
<td>0.01±0.04</td>
</tr>
</tbody>
</table>

SNP indicates sodium nitroprusside.

Data are presented as mean±SD.

Drug doses are in µg·100 mL tissue⁻¹·min⁻¹. tPA values are in ng/mL.

*P<0.05 vs normotensive subjects; †P<0.05 vs baseline.

Table 2. Arterial, Venous, and Venous-Arterial Concentration Gradient of tPA at Baseline and After Infusion of Bradykinin and Sodium Nitroprusside

Figure 2. Effect of bradykinin on tPA release in the presence of saline (open bars) or sulfaphenazole (solid bars) in normotensive subjects (A) and in patients with essential hypertension (B). Data are mean±SD. *P<0.01 vs baseline; †P<0.01 vs bradykinin plus saline.
in hypertensive patients was confirmed (Figures 3 and 4). As expected, in normotensive subjects, the vascular response to both bradykinin (Figure 3A) and acetylcholine (Figure 4A) was significantly reduced by L-NMMA. In these subjects, sulfaphenazole administration, which did not significantly change basal FBF, was devoid of effect on response to bradykinin (Figure 3A) or to acetylcholine (Figure 4A). When L-NMMA was infused with sulfaphenazole, a further reduction in response to bradykinin (Figure 3A), but not to acetylcholine (Figure 4A), was observed.

As expected, in hypertensive patients, vasodilation in response to bradykinin was resistant to L-NMMA and was significantly reduced by sulfaphenazole (Figure 3B). A similar reduced response to bradykinin was observed in the presence of L-NMMA and sulfaphenazole coinusions (Figure 3B). The residual vasodilation response to bradykinin with simultaneous L-NMMA and sulfaphenazole administration was similar in both normotensive subjects (Figure 3A) and hypertensive patients (Figure 3B). By contrast, vasodilation in response to acetylcholine, which was unaffected by L-NMMA, was resistant to sulfaphenazole and to L-NMMA and sulfaphenazole coadministration (Figure 4B).

As already observed, in normotensive subjects, bradykinin-induced and acetylcholine-induced tPA release was significantly ($P<0.01$) higher than in hypertensive patients (Figures 5 and 6). In this group of normotensive subjects, the presence of L-NMMA significantly decreased the tPA release induced by both bradykinin (Figure 5A) and acetylcholine (Figure 6A). However, whereas sulfaphenazole significantly reduced bradykinin-stimulated tPA release (Figure 5A), no inhibitory effect was observed on acetylcholine-induced tPA release (Figure 6A). When L-NMMA and sulfaphenazole were infused simultaneously, although the bradykinin-induced tPA release was almost abolished (Figure 5A), no further effect was observed on acetylcholine-induced tPA release (Figure 6B). In hypertensive patients, sulfaphenazole but not...
L-NMMA significantly blunted bradykinin-induced tPA release (Figure 5B) but not tPA release induced by acetylcholine (Figure 6B). Finally, when simultaneously infused, L-NMMA and sulfaphenazole induced a reduction in tPA release similar to sulfaphenazole alone after bradykinin (Figure 5B) but not acetylcholine infusion (Figure 6B). In both groups, contralateral FBF and venous-arterial concentrations of tPA were unchanged throughout each protocol (data not shown).

**Discussion**

As reported previously, the present results confirm the presence of a blunted response to bradykinin and acetylcholine in hypertensive patients compared with normotensive subjects. The present results also confirm that although in healthy conditions, the response to bradykinin is sensitive to the NO synthase inhibitor L-NMMA, in essential hypertension, the residual vasodilation response to the endothelial agonist is resistant to NO blockade but is sensitive to sulfaphenazole, an in vitro specific inhibitor of CYP 2C9.

By contrast, the vasodilation response to acetylcholine, which was blunted by NO inhibition in normotensive subjects but not in hypertensive patients, was resistant to sulfaphenazole. These findings do not support the hypothesis that NO-independent vasodilation in response to acetylcholine is mediated by a CYP 2C9–dependent pathway in these experimental conditions, in line with previous findings. However, given that a CYP 2C isoform is involved in acetylcholine-induced EDHF generation in hamster gracilis muscle, a role of CYP 2C9 cannot be ruled out. It is conceivable that the CYP 2C9-dependent vasodilation response to acetylcholine, which might act as a weak CYP 2C9 activator, is detectable at a higher infusion rate of acetylcholine.

It is of interest that the inhibitory effect of sulfaphenazole on vasodilation in response to bradykinin was detectable in normotensive subjects only when NO availability was abolished by concomitant L-NMMA administration. Therefore, these findings reinforce the concept that although in healthy conditions, the NO pathway represents the main mechanism that accounts for vasodilation in response to bradykinin,
under conditions characterized by impaired NO availability, including essential hypertension, an alternative acute compensatory pathway, possibly CYP 2C9 dependent, can be detected.15

The major new finding of the present study is related to the mechanisms underlying bradykinin-stimulated tPA release in humans. As reported previously,24,25 bradykinin induces a significant increase of tPA release in healthy subjects. This effect is specific and not flow dependent, because it was not detected when infusion of the endothelium-independent relaxing compound sodium nitroprusside was used in the same experimental conditions. In addition, bradykinin-mediated tPA release was found to be sensitive to L-NMMA, which indicates a positive modulatory effect of the NO pathway in physiological conditions, a finding in line with previous reports of application of different endothelial stimuli, such as substance P or epinephrine.3,26 The positive role of the NO pathway was further confirmed by the finding that acetylcholine-induced tPA release was significantly blunted by NO inhibition in normotensive subjects.5

In normotensive subjects, sulfaphenazole also significantly reduced bradykinin-induced tPA release, a finding that suggests that a CYP 2C9–dependent pathway could be physiologically able to participate in fibrinolysis modulation. This possibility agrees with experimental evidence indicating that bradykinin-stimulation of CYP 2C9 is able to release EETs,10,22 which in turn promote the induction of tPA expression in endothelial cells.15 Accordingly, in cultured human arterial endothelial cells, thrombin-induced tPA release was found to be mediated by EETs.16 Of particular note was the finding that in the presence of simultaneous infusion of L-NMMA and sulfaphenazole, bradykinin-stimulated tPA release was further reduced, which demonstrates that the 2 antagonists act on different but complementary pathways. The present findings suggest that the effect of bradykinin is specific, because tPA release by acetylcholine was unaffected by sulfaphenazole either in the absence or presence of NO inhibition. Finally, this concept is further confirmed because the coinfusion of sulfaphenazole failed to affect the response to sodium nitroprusside.

In hypertensive patients, bradykinin-induced and acetylcholine-induced tPA release was impaired, a finding in line with a well-documented reduction in endothelial fibrinolytic capacity in this clinical condition, previously demonstrated with different stimuli, including desmopressin,27 substance P,28 acetylcholine,4 and epinephrine.26 It is, however, interesting to observe that in hypertensive patients, the residual but still evident bradykinin-induced tPA release was totally resistant to L-NMMA, whereas it was blocked by sulfaphenazole. Finally, when L-NMMA was coinfused with sulfaphenazole, no further reduction in tPA release was observed. In contrast, in hypertensive patients, tPA release by acetylcholine was totally resistant to both L-NMMA and sulfaphenazole, and no further effect was detectable with L-NMMA and sulfaphenazole coinfusion.

Taken together, these findings suggest that in essential hypertension, the impairment of NO availability leads to a reduction in fibrinolytic capacity, and residual tPA release in response to bradykinin but not to acetylcholine could depend on a CYP 2C9–related pathway, sensitive to sulfaphenazole, possibly via an EDHF identified with EETs.4,27,28 The finding that acetylcholine-induced tPA release was resistant to the inhibitory effect of sulfaphenazole further reinforces the concept that the effect of sulfaphenazole on bradykinin-mediated tPA release is specific. The present results demonstrate that in physiological conditions, the NO and EDHF pathways appear to be equally involved in the modulation of bradykinin-stimulated tPA release, whereas vascular responses appear to be mediated almost exclusively by NO. The different mechanism involved in vascular and fibrinolytic responses needs to be explored further. The possibility exists that smooth muscle cells, when stimulated by NO, are no longer sensitive to hyperpolarization, whereas tPA release is still sensitive to both pathways. This hypothesis is confirmed by the finding that when NO production is blocked by L-NMMA, the inhibitory effect of sulfaphenazole, most likely related to the inhibition of CYP 2C9, becomes detectable. Furthermore, in essential hypertension, and therefore in the presence of impaired NO availability, the effect of sulfaphenazole suggests that a CYP 2C9–dependent pathway can operate as a “residual” mechanism responsible for endothelium-dependent vasodilation and modulation of fibrinolysis. The present findings do not support the possibility that in patients with essential hypertension, activation of the CYP 2C9–dependent pathway could be a significant source of oxygen-derived free radicals, as reported in patients with coronary artery disease.29 This discrepancy could be related to the different degree of cardiovascular risk that characterizes the 2 study populations. Because it was demonstrated that the level of endothelial dysfunction is related to total cardiovascular risk, a shifting of CYP 2C9 from production of oxygen-derived free radicals in the presence of coronary artery disease is conceivable. This effect could account for the vasodilatory effect of sulfaphenazole observed in patients with coronary artery disease.29

A major limitation of the present study concerns the lack of a direct demonstration of an effective inhibitory effect of sulfaphenazole on CYP 2C9 under the experimental conditions studied. Previous findings conducted in porcine coronary arteries30 demonstrated that CYP 2C9 expressed in vascular endothelium11,32 could be inhibited by sulfaphenazole, a highly selective inhibitor of CYP 2C9.17,18,33 These data confirm a link between endothelial CYP 2C9 activity and the generation of EETs acting as EDHFs.22

The results of the present study show that sulfaphenazole significantly blunts NO-independent vasodilation in response to bradykinin infusion in hypertensive patients, which suggests that CYP 2C9-derived EETs act as EDHFs, as previously reported under the same experimental conditions.13 However, because effective inhibition of CYP2C9 under sulfaphenazole infusion has not been demonstrated in the present experimental conditions, the specific role of CYP 2C9 in modulating tPA release, although conceivable, must be better characterized.

Another limitation of the present study concerns the slight difference in age of the 2 study populations. The effect of age on endothelial function is similar to that of essential hypertension, and the aging process actually amplifies the effect of
essential hypertension on NO availability.\textsuperscript{34} Given that the NO pathway mainly promotes tPA release in healthy endothelium, a similar impact of aging on tPA release in hypertensive patients is conceivable; however, this attractive hypothesis must be explored further.

In conclusion, the results of the present study may provide additional information concerning the pathways involved in the modulation of acute tPA release in humans. Because endothelial fibrinolytic capacity, in addition to endothelium-dependent vasodilation,\textsuperscript{1} may predict the risk of future cardiovascular events,\textsuperscript{35} an understanding of the pathways involved in the reduced fibrinolytic potential might provide future insights for determination of cardiovascular risk in essential hypertension. In addition, restoration of endothelial fibrinolytic properties might become an adjunctive target of antihypertensive therapy. Thus, identification of the pathways that characterize impaired tPA release can increase the knowledge base of the pathophysiology of atherosclerotic disease and provide additional potential for the development of specific strategies to improve endothelial dysfunction.

Acknowledgments

The authors gratefully acknowledge Dr M. Urooj Zafar for his help in the revision of the manuscript.

Disclosures

None.

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**CLINICAL PERSPECTIVE**

Despite the arterial wall stress caused by high blood pressure, patients with essential hypertension are paradoxically more exposed to thrombotic (ie, acute myocardial infarct and ischemic stroke) rather than hemorrhagic complications. It is conceivable that the dysfunctional endothelium, an early vascular alteration in hypertension, and other classic risk factors might be the promoter of the increased atherothrombotic risk. In line with this possibility, recent findings suggest that in addition to the regulation of vascular tone, abnormal endothelium-derived regulation of endogenous fibrinolysis could account for the atherothrombotic complications that characterize essential hypertension. Accordingly, the release of tissue plasminogen activator (tPA) has been proposed recently as a new and distinct marker of endothelial function in humans. The results of the present study show that in healthy conditions, the release of tPA induced by the endothelial agonist bradykinin depends on the activation of both NO and NO-independent pathways. In hypertensive patients, tPA release is reduced because of the impaired NO availability, whereas the residual tPA release is only sustained by NO-independent mechanisms. Interestingly, NO-independent, bradykinin-induced tPA release can be blocked by sulfaphenazole, a compound that in vitro blocks the activity of cytochrome P450 epoxygenase (CYP 2C9), a well-documented source of hyperpolarizing factor. Thus, the possibility exists that in hypertensive patients, the reduced dynamic tPA release from vascular endothelium could be part of a generalized endothelial dysfunction. This alteration may contribute to the hypofibrinolytic state that characterizes this clinical condition and possibly represents a more specific therapeutic target to improve endothelial fibrinolytic function and reduce cardiovascular risk in essential hypertension.
Effect of Sulfaphenazole on Tissue Plasminogen Activator Release in Normotensive Subjects and Hypertensive Patients
Chiara Giannarelli, Agostino Virdis, Ferdinando De Negri, Armando Magagna, Emiliano Duranti, Antonio Salvetti and Stefano Taddei

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Table 2 of the article reports the arterial, venous and arteriovenous concentration gradient of tissue-type plasminogen activator (t-PA) at baseline and after infusion of bradykinin and sodium nitroprusside. The above-mentioned concentrations of t-PA should be ng/mL instead of ng · min⁻¹ · 100 mL⁻¹ as inappropriately reported in the Table. This was a typographical error, and the calculation of t-PA release had been properly performed as reported in the Methods section. The Table, reproduced below, has been corrected in the current online version of the article.

The authors regret the error.

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**Table 2. Arterial, Venous, and Venous-Arterial Concentration Gradient of t-PA at Baseline and After the Infusion of Bradykinin and Sodium Nitroprusside**

<table>
<thead>
<tr>
<th>Drug Dose</th>
<th>Normotensive Subjects (n=22)</th>
<th>Hypertensive Patients (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arterial t-PA</td>
<td>Venous t-PA</td>
</tr>
<tr>
<td>Baseline</td>
<td>1.31±0.35</td>
<td>1.36±0.35</td>
</tr>
<tr>
<td>BDK (0.015)</td>
<td>1.33±0.36</td>
<td>1.46±0.38†</td>
</tr>
<tr>
<td>Baseline</td>
<td>1.35±0.34</td>
<td>1.36±0.33</td>
</tr>
<tr>
<td>SNP (0.2)</td>
<td>1.34±0.35</td>
<td>1.35±0.32</td>
</tr>
</tbody>
</table>

SNP indicates sodium nitroprusside.  
Data are presented as mean±SD.  
Drug doses are in μg · 100 mL forearm tissue⁻¹ · min⁻¹. tPA values are in ng/mL.  
*P<0.05 vs normotensive subjects; †P<0.05 vs baseline.