Smoking Impairs Bradykinin-Stimulated t-PA Release

Mias Pretorius, David A. Rosenbaum, Jean Lefebvre, Douglas E. Vaughan, Nancy J. Brown

Abstract—Bradykinin stimulates tissue plasminogen activator release from human endothelium through a flow-independent, B2 receptor–dependent mechanism. The present study tests the hypothesis that smoking impairs bradykinin-stimulated tissue plasminogen activator release. Graded doses of nitroprusside (1.6 to 6.4 μg/min), methacholine (3.2 to 12.8 μg/min), and bradykinin (100 to 400 ng/min) were infused in the brachial artery in random order in 20 smokers and 12 nonsmokers matched for age, gender, and body mass index. Forearm blood flow was measured by strain-gauge plethysmography. All 3 drugs caused a dose-dependent increase in forearm blood flow, with no significant difference between smokers and nonsmokers. Bradykinin (P=0.001) and methacholine (P=0.001) caused significant dose-dependent increases in net tissue plasminogen activator release. The tissue plasminogen activator response to bradykinin was significantly greater than the tissue plasminogen activator response to methacholine in the nonsmokers (maximal net tissue plasminogen activator release, 73.2±21.5 versus 27.6±7.2 ng/min per 100 mL; P=0.001) but not in the smokers (maximal net tissue plasminogen activator release, 44.5±10.7 versus 24.8±9.3 ng/min per 100 mL; P=0.154). The effect of bradykinin (P=0.037), but not methacholine (P=0.978), on net tissue plasminogen activator release was significantly reduced in smokers compared with nonsmokers. The vascular tissue plasminogen activator response to bradykinin, but not methacholine, is impaired in smokers.

Stimulated tissue plasminogen activator release may be a more sensitive measure of endothelial function than vasodilation. (Hypertension. 2002;39:767-771.)

Key Words: bradykinin • t-PA release • smoking • endothelium • plethysmography

A cute rupture of a coronary atheromatous plaque and subsequent coronary thrombosis play a critical role in the pathogenesis of myocardial infarction and sudden cardiac death.1 The prevention of thrombosis in the coronary and other vascular beds is heavily dependent on the vascular endothelium. The endothelium generates and secretes short-lived potent platelet inhibitors, including NO and prostacyclin.2 The endothelial receptor thrombomodulin promotes the thrombin-dependent activation of protein C, which in turn inhibits coagulation factors V and VIII.3 The endothelium contributes to fibrinolysis by synthesizing, storing, and secreting tissue-type plasminogen activator (t-PA) and by expressing receptors that bind t-PA and enhance its activity.4 It has been suggested that the plasminogen activator system serves as one of the major endogenous defenses against coronary thrombosis.5 Although studies of endothelial function in humans have focused on the vasodilatory capacity of the endothelium, the effect of risk factors for coronary artery disease on the fibrinolytic capacity of the endothelium has been less well characterized. In hypertensive subjects, Jern and coworkers6 have reported decreased desmopressin-stimulated, but not methacholine-stimulated, release of t-PA activity and antigen across the forearm compared with that of normal controls. Newby et al7 reported decreased venous t-PA antigen concentrations and activity and impaired vasodilation after intra-brachial artery infusion of substance P in smokers compared with nonsmokers. Similarly, this group has observed impaired coronary release of active t-PA in response to substance P in smokers compared with nonsmokers.8 Bradykinin is a potent stimulus to t-PA synthesis and release in endothelial cells9 and in perfused tissue preparations such as the pig ear or the rat hind limb.10,11 Bradykinin stimulates t-PA release from the human forearm and coronary vasculature in a dose-dependent fashion.12,13 In addition, endogenous bradykinin contributes to many of the cardioprotective effects of ACE inhibitors,14 and ACE inhibition potentiates both the vasodilator and the t-PA responses to exogenous bradykinin.13,15 Despite the potential clinical relevance of bradykinin-stimulated t-PA release, studies elucidating the effect of risk factors for coronary artery disease on bradykinin-stimulated t-PA release do not exist.

Cigarette smoking is a major risk factor for the development of atherosclerosis and thrombotic events.16,17 The purpose of this study was to test the hypothesis that cigarette smoking is associated with impaired endothelial t-PA release in response to bradykinin. The forearm vasodilator and t-PA response to bradykinin was compared with responses to...
methacholine (an endothelium-dependent vasodilator) and nitroprusside (an endothelium-independent vasodilator).

Methods

Subjects

The study was approved by the Vanderbilt University Institutional Review Board and conducted according to the Declaration of Helsinki, with the written informed consent from each subject. Subjects with significant cardiovascular, renal, pulmonary, endocrine, or hematological disease and pregnant women were excluded. Subjects were within 30% of their ideal body weight and had a fasting total serum cholesterol $<5.69$ mmol/L (220 mg/dL). Subjects were defined as smokers if they smoked 5 to 25 cigarettes per day. Studies were not initiated unless the 24-hour urine sodium excretion was between 100 and 200 mmol.

Experimental Protocol

Subjects were studied in the fasting state and refrained from smoking on the morning of the study. An intravenous catheter was placed in the antecubital vein, and an 18-gauge polyurethane catheter (Cook Inc) was inserted into the brachial artery of the nondominant arm. Before and between the infusion of drugs, arterial catheter patency was maintained by infusion of 5% dextrose at 1 mL/min for 30 minutes before baseline measurements were made.

After measurement of basal forearm blood flow (FFB) and blood sampling, sodium nitroprusside, methacholine, or bradykinin was infused in random order. Sodium nitroprusside was infused at 1.6, 3.2, and 6.4 μg/min; methacholine at 3.2, 6.4, and 12.8 μg/min; and bradykinin at 100, 200, and 400 ng/min. Each dose was infused for 5 minutes, and FFB was measured during the last 2 minutes. Drug concentrations were adjusted to maintain infusion volumes at 1 mL/min. One nonsmoker inadvertently received vehicle during the methacholine infusion, and those data were not included in the analysis.

Forearm Perfusion Measurements and Blood Sampling

FFB was measured using mercury-in-silastic strain gauge plethysmography, as previously described. After measurement of FFB, simultaneous arterial and venous samples were obtained from the infused arm before and after each dose of bradykinin and methacholine for measurement of t-PA and plasminogen activator inhibitor-1 (PAI-1). During bradykinin infusions, samples were also obtained for measurement of the bradykinin metabolite BK$_{1-6}$. All samples were obtained after the first 3 mL of blood were discarded. Blood samples were collected on ice and centrifuged immediately, and plasma was stored at −70°C until assay.

Biochemical Assays

Blood for measurement of PAI-1 and t-PA was collected in tubes containing 0.105 mol/L sodium citrate. Antigen levels were determined using a 2-site enzyme-linked immunosorbent assay (Biopool AB). Individual net release or uptake rates at each time point were calculated using the formula: net release=[(C$_0$ − C$_i$)×FFB]. We have previously demonstrated that changes in t-PA activity parallel changes in t-PA antigen, and therefore, we measured only t-PA antigen concentrations here. Blood for measurement of BK$_{1-6}$ was drawn into cold anhydrous ethanol. BK$_{1-6}$ was determined using a dual-isotope dilution mass spectroscopic assay as previously described.

Statistics

Data are presented as mean±SEM in the Figures and mean±SD in the Table. Categorical data were compared using χ$^2$ or Fischer’s exact tests. Comparisons between groups were made using a general linear model-repeated measures ANOVA in which the within subject variable was drug and/or dose and the between subject variable was smoking status. Post hoc comparisons were made using the paired t test or Wilcoxon signed rank test, as appropriate. A 2-tailed P value <0.05 was considered significant. All analyses were performed using SPSS for Windows (version 10.0).

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results

Demographics

The Table provides the clinical characteristics of the 20 smokers and 12 nonsmoker controls who participated in the study. There were no significant differences between the 2 groups with regard to age, gender, race, body mass index, blood pressure, cholesterol, baseline FFB, or baseline net t-PA release. Resting heart rate was significantly higher in smokers compared with nonsmokers.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nonsmokers (n=12)</th>
<th>Smokers (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>9 (75%)</td>
<td>16 (80%)</td>
<td>1.0</td>
</tr>
<tr>
<td>African American</td>
<td>3 (25%)</td>
<td>4 (20%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Female</td>
<td>6 (50%)</td>
<td>10 (50%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Age, y</td>
<td>33.5±8.6</td>
<td>35.5±9.9</td>
<td>0.577</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.4±2.4</td>
<td>25.3±4.3</td>
<td>0.526</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>65±15</td>
<td>76±12</td>
<td>0.032</td>
</tr>
<tr>
<td>s-BP, mm Hg</td>
<td>114±11</td>
<td>114±11</td>
<td>0.994</td>
</tr>
<tr>
<td>d-BP, mm Hg</td>
<td>65±8</td>
<td>67±6</td>
<td>0.325</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>81±7</td>
<td>83±7</td>
<td>0.506</td>
</tr>
<tr>
<td>Cholesterol mmol/L, mg/dL</td>
<td>4.5±0.5</td>
<td>4.7±0.6</td>
<td>0.347</td>
</tr>
<tr>
<td>(173.8±19.5)</td>
<td>(181.5±22.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline FFB, mL/min/100 mL</td>
<td>4.06±1.45</td>
<td>4.65±2.51</td>
<td>0.464</td>
</tr>
<tr>
<td>Baseline net-tPA release, ng/min/100 mL</td>
<td>0.42±1.36</td>
<td>0.03±1.92</td>
<td>0.735</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SD. BMI indicates body mass index; s-BP, systolic blood pressure; d-BP, diastolic blood pressure; MAP, mean arterial pressure; FFB, forearm blood flow; t-PA, tissue plasminogen activator.
Hemodynamics and FBF

There was no effect of any agonist on systemic mean arterial pressure; therefore, data are presented as FBF. Nitroprusside (P<0.001), methacholine (P<0.001), and bradykinin (P<0.001) increased FBF in a dose-dependent fashion (Figure 1). In both smokers and nonsmokers, the increase in FBF in response to methacholine was significantly greater than the increase in FBF in response to either nitroprusside (P=0.001) or bradykinin (P=0.001). There was no significant difference between smokers and nonsmokers in the FBF response to nitroprusside (P=0.460), methacholine (P=0.99), or bradykinin (P=0.534). Forearm venous BK1−5 concentrations increased significantly during intraarterial infusion of bradykinin (P<0.001), and there was no significant effect of smoking on kinin levels (venous BK1−5 concentrations during 400 ng/min bradykinin infusion, 451.2±55.8 versus 659.5±167.4 fmol/mL in smokers versus nonsmokers; P=0.167). When BK1−5 concentration response curves were derived for each subject, there was no difference in the slopes of the concentration-FBF curve or concentration–net t-PA release curve between smokers and nonsmokers (both, P>0.290).

Fibrinolytic Parameters

Net t-PA release increased in a dose-dependent fashion in response to bradykinin (from 0.5±0.4 to 73.2±21.5 ng/min per 100 mL in nonsmokers, P<0.001) and methacholine (from 0.2±0.7 to 27.6±7.2 ng/min per 100 mL in nonsmokers P<0.001) (Figure 2). The increase in net t-PA release in response to bradykinin was significantly decreased in smokers (to 44.5±10.7 ng/min per 100 mL, P=0.037) compared with nonsmokers. In contrast, there was no effect of smoking on the t-PA response to methacholine (24.8±9.3 ng/min per 100 mL, P=0.978). The t-PA response to bradykinin was significantly greater than the t-PA response to methacholine in the nonsmokers (P<0.001) but not in the smokers (P=0.154). As reported previously, there was no effect of either bradykinin or methacholine on PAI-1 antigen (data not shown).

Discussion

In this study, we compared the vasodilator and t-PA responses to nitroprusside, methacholine, and bradykinin in healthy smokers and nonsmokers. The data indicate that the fibrinolytic response to bradykinin, a peptide that contributes to many of the cardioprotective effects of ACE inhibitors, is significantly impaired in cigarette smokers. In contrast to the vasodilator response, which was not affected by smoking, the t-PA response to bradykinin was significantly decreased in smokers compared with nonsmokers. These findings suggest a potential role for cigarette smoking in the development of cardiovascular disease, particularly with regard to the fibrinolytic pathway.
methacholine and bradykinin or to the endothelium-independent agonist nitroprusside. This contrasts with several previous reports that endothelium-dependent vasodilation is impaired in smokers\textsuperscript{7,21,22} compared with nonsmokers, but is consistent with other studies\textsuperscript{23–25} that found no effect of smoking on endothelium-dependent vasodilation. The reasons underlying the diverse findings with respect to the effect of cigarette smoking on endothelial vasodilator function are not certain but may involve confounding genetic and environmental influences. Data from Celemajer and coworkers\textsuperscript{26} suggests that endothelial dysfunction in smokers may be dose dependent; these investigators reported an inverse relationship between pack-years of cigarette exposure and flow-mediated vasodilation. In this regard, the mean age (and therefore the duration of exposure) of the smokers studied in the present study was younger than that reported in several previously published studies.\textsuperscript{8,21} In addition, Heitzer et al\textsuperscript{21} have demonstrated a synergistic effect of hypercholesterolemia and cigarette smoking on endothelium-dependent vasodilation. The exclusion of subjects with hyperlipidemia may have minimized the effect of cigarette smoking on endothelium-dependent vasodilation in the present study.

In contrast to the lack of smoking on endothelium-dependent vasodilation, smoking was associated with an impaired t-PA response to bradykinin. This is consistent with 2 earlier studies\textsuperscript{7,8} that demonstrated that the t-PA response to intrabrachial and intracoronary infusion of substance P is also impaired in smokers. Similarly, Allen et al\textsuperscript{21} have reported a diminished t-PA response to systemic infusion of desmopressin in smokers compared with nonsmokers. In the present study, the finding that the fibrinolytic response to bradykinin is impaired and that the vasodilator response remains intact in smokers suggests that bradykinin causes vasodilation and t-PA release through different mechanisms. In support of this, bradykinin stimulates t-PA release through a NO synthase– and cyclooxygenase-independent pathway,\textsuperscript{28} whereas inhibition of NO synthase attenuates the vasodilator response to bradykinin.

An important difference between this study and previous studies examining the effect of smoking on the t-PA response to substance P\textsuperscript{7} or desmopressin\textsuperscript{27} is the inclusion of an endothelium-dependent control. The muscarinic agonist methacholine has been shown to increase net release of t-PA across the forearm of both normotensive and hypertensive subjects.\textsuperscript{29,30} In the current study, the t-PA response to methacholine was significantly less than that of bradykinin in the nonsmokers, even though the flow response was significantly greater. Moreover, there was no effect of smoking on the t-PA response to methacholine, such that the t-PA response to bradykinin and methacholine were statistically comparable in smokers. Similarly, Jern and co-workers\textsuperscript{6,29,31} have reported that the t-PA response to intraarterial methacholine is preserved in patients with hypertension and diabetes, whereas the t-PA response to intraarterial desmopressin is impaired in hypertension.

The reason for the differential effect of smoking on methacholine- and bradykinin-stimulated t-PA release remains to be determined. The achievement of similar kinin concentrations in the smokers and nonsmokers during intraarterial bradykinin infusion excludes a simple pharmacokinetic explanation for the differential effect of smoking on the t-PA response to the 2 secretagogues. To the extent that conversion of bradykinin to BK\textsubscript{1,2} reflects serum ACE activity in humans,\textsuperscript{20} smoking does not appear to affect ACE activity. In addition, the lack of effect of smoking on the relationship between kinin concentrations and either FBF or net t-PA release does not support an effect of smoking on BK\textsubscript{1,2} receptor sensitivity. Although bradykinin and methacholine act through unique receptors (B\textsubscript{2} and M\textsubscript{3}, respectively), both agonists act through G\textsubscript{q} to activate phospholipase C and to stimulate the production of inositol triphosphate.\textsuperscript{32–33} Both bradykinin\textsuperscript{34} and methacholine\textsuperscript{35} stimulate the release of endothelium-derived hyperpolarizing factor, as well as NO and prostacyclin. Taken together with the observation that bradykinin stimulates t-PA release through a NO synthase– and cyclooxygenase-independent pathway,\textsuperscript{28} the finding that smoking blunts bradykinin-stimulated, but not methacholine-stimulated, t-PA release suggests that bradykinin stimulates t-PA release in part through a unique as-yet-to-be-identified pathway.

Although the perfused forearm serves as a convenient model for studying the effect of smoking on bradykinin-stimulated t-PA release, the use of this system limits the potential applicability of our findings to the coronary vasculature. However, Minai et al\textsuperscript{13} have demonstrated that bradykinin stimulates t-PA release from the coronary vasculature to a similar extent as what we have observed in the peripheral vasculature. In addition, the studies of Newby et al\textsuperscript{7,8} suggest that the effect of smoking on forearm endothelial t-PA release parallels the effect of smoking on the endothelial fibrinolytic function of the coronary vasculature. Nevertheless, it is not possible to extrapolate our findings regarding the effect of smoking on bradykinin-stimulated t-PA release to the coronary vasculature.

In summary, this is the first study to demonstrate that bradykinin-stimulated t-PA release is attenuated in smokers compared with nonsmokers. To the extent that endogenous bradykinin contributes to many of the effects of ACE inhibitors,\textsuperscript{8} this study suggests the hypothesis that smoking affects the fibrinolytic response to ACE inhibition. In addition, the finding that bradykinin-stimulated t-PA release is compromised in smokers, although the vasodilator response remains intact, suggests that the fibrinolytic response to bradykinin provides a more sensitive measure of early endothelial dysfunction than the vasodilator response. Further studies are needed to examine the quantitative relationship between cigarette exposure and bradykinin-stimulated t-PA release and the prognostic implication of impaired vascular t-PA release.

Acknowledgments

We would like to thank Tami Neal, RN, for her nursing assistance and Dr Ru Jiao Shan for her technical assistance. This work was funded by National Institutes of Health grants HL 65193, HL 60906, and RR 00095.

References

Smoking Impairs Bradykinin-Stimulated t-PA Release
Mias Pretorius, David A. Rosenbaum, Jean Lefebvre, Douglas E. Vaughan and Nancy J. Brown

Hypertension. 2002;39:767-771
doi: 10.1161/hy0302.105767

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/39/3/767

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2002/03/03/39.3.767.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located,
click Request Permissions in the middle column of the Web page under Services. Further information about
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/