Angiotensin-Converting Enzyme Insertion/Deletion Polymorphism Modulates the Human In Vivo Metabolism of Bradykinin

Laine J. Murphey, MD, PhD; James V. Gainer, MD; Douglas E. Vaughan, MD; Nancy J. Brown, MD

Background—Bradykinin is a cardioprotective peptide metabolized by the angiotensin-converting enzyme (ACE). An insertion/deletion (I/D) polymorphism in the ACE gene determines plasma ACE levels. The D allele is associated with cardiovascular disease, which may relate to increased angiotensin II production or to increased bradykinin degradation to the inactive metabolite bradykinin 1–5 (BK1–5). Therefore, we determined the effect of the ACE I/D polymorphism on human bradykinin metabolism in vivo.

Methods and Results—Bradykinin (400 ng/min) was infused into the brachial artery of volunteers with ACE I/I, I/D, or D/D genotypes (n=9 each). The bradykinin and BK1–5 levels in forearm venous return were quantified by liquid chromatography–mass spectrometry. Plasma ACE activity was highest in those with the D/D genotype (36.8±6.2 U/mL), intermediate in those with the I/D genotype (25.3±3.3 U/mL), and lowest in those with the I/I genotype (20.3±2.3 U/mL; \( P=0.017 \) for effect of number of D alleles). Bradykinin concentrations were 726±242, 469±50, and 545±104 fmol/mL in I/I, I/D, and D/D subjects, respectively (\( P=0.10 \)). Significant correlations existed between the number of D alleles and BK1–5 concentrations (1113±290, 1520±318, and 1887±388 fmol/mL in the I/I, I/D, and D/D groups, respectively; \( P=0.027 \)) and the ratio of BK1–5 to bradykinin (1.87±0.35, 3.09±0.40, and 4.31±0.97 in the I/I, I/D, and D/D volunteers, respectively; \( P=0.010 \)). The venous blood BK1–5:bradykinin ratio correlated with plasma ACE activity (\( r^2=0.16, P=0.039 \)), and total kinin concentration correlated with net tissue plasminogen activator release across the forearm (\( r^2=0.20, P=0.027 \)).

Conclusions—The ACE D allele has a significant effect on the in vivo degradation of bradykinin in humans. The ratio of BK1–5:bradykinin may serve as a marker for tissue ACE activity. (Circulation. 2000;102:829-832.)

Key Words: bradykinin • metalloproteinases • metabolism • endothelium

A ngiotensin-converting enzyme (ACE) catalyzes the conversion of angiotensin I to angiotensin II and the degradation of bradykinin to inactive products.1 An insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene accounts for 50% of the variability of human serum ACE levels.2 The D allele is associated with increased ACE activity and has been linked with cardiovascular disease.3 This link has been attributed to the increased formation of angiotensin II in individuals who carry the D allele.4 A complimentary hypothesis is that cardiovascular morbidity associated with the D allele reflects an increased degradation of cardioprotective bradykinin.

Bradykinin is a potent vasodilator that exerts antiproliferative effects, inhibits thrombin-induced platelet activation, contributes to the cardioprotective effects of ACE inhibitors,5 and stimulates tissue-type plasminogen activator (t-PA) release from vascular endothelium.6 We previously reported that the ACE genotype determines the rate of bradykinin degradation in human sera ex vivo.7 However, serum ACE activity represents only a small fraction of the in vivo activity of this endothelium-bound enzyme.1

Campbell et al8 used the ratio of bradykinin 1–7 (BK1–7) to bradykinin to measure bradykinin degradation by ACE. Recently, we determined the human metabolism of systemic bradykinin in vivo and identified BK1–5 (Arg-Pro-Pro-Gly-Phe) as a stable, circulating metabolite.9 Whereas BK1–7 represents the product of a single cleavage of bradykinin by ACE, BK1–5 is produced by 2 sequential cleavages at the Pro7-Phe8 and Phe5-Ser6 bonds. Thus, the ratio of BK1–5 to bradykinin in the circulation may reflect the sum of vascular, endothelial, and serum ACE activity. Therefore, we ascertained the effect of ACE genotype on the metabolism of intra-arterially administered bradykinin in the human forearm vasculature.

Methods

Subjects
A total of 27 normotensive volunteers with the ACE I/I, I/D, or D/D genotype (n=9 each) were studied. The protocol was approved by...
the Vanderbilt University Institutional Review Board and conducted according to institutional guidelines. All volunteers gave written, informed consent and underwent a history, physical examination, laboratory analysis, and ECG. All were healthy nonsmokers participating in ongoing studies of the effects of race and sodium intake on vasodilator responses.

**Experimental Protocol**

Subjects were studied under salt-replete conditions, as previously described. Briefly, catheters were inserted into the brachial artery and antecubital vein of the nondominant arm for intra-arterial bradykinin infusion and venous sampling. Bradykinin (Calbiochem; purified and pyrogen-tested by the Vanderbilt Investigational Pharmacy) was infused at 100, 200, and 400 ng/min for 5 minutes each. The forearm blood flow (FBF) response was determined using strain-gauge plethysmography, and net t-PA release was calculated as the product of the arteriovenous t-PA concentration gradient and forearm plasma flow, as reported elsewhere. During the 400 ng/min infusion only, venous blood for kinins (5 mL) was drawn through the intravenous catheter and immediately added to 15 mL of chilled ethanol to inhibit ex vivo bradykinin formation. Samples were centrifuged at 4°C for 20 minutes, and the supernatant was stored at −70°C until analysis.

**Bradykinin and BK1–5 Analysis**

Bradykinin and its metabolite BK1–5 were analyzed by liquid-chromatography electrospray mass-spectrometry, as described previously. Briefly, internal standards ([2H8-Phe5]bradykinin and [13C2,15N-Gly4]BK1–5), synthesized by Dr James Elliott, Yale University, New Haven, Conn) were added to 5 mL of ethanolic plasma supernatant, dried under nitrogen at 37°C, and extracted on a C-18 Sep-Pak cartridge (Waters). Gradient chromatography on an Eclipse XDB-C18 column (2.1×50 mm, 5-μm particle, Hewlett-Packard) was coupled to a FinniganMAT TSQ7000-series triple-quadrupole mass spectrometer. Parent ions (bradykinin m/z 531 [M+2H]2+ and BK1–5 m/z 287 [M+2H]2+) underwent collision-induced dissociation at −34 and −14 eV voltage offset, respectively, with daughter ions m/z 270 (bradykinin) and m/z 408 (BK1–5) monitored. For internal standards, the transitions were m/z 553 to m/z 270 ([2H8-Phe5]bradykinin) and m/z 288.5 to m/z 411 ([13C2,15N-Gly4]BK1–5). Peptides were quantified by comparing the signal strengths of unknowns with coanalyzed internal standards, corrected for dilution in ethanol, and reported per milliliter of blood collected.

**Genotyping and Biochemical Assays**

The ACE I/D genotype was determined using polymerase chain reaction, with D/D genotypes confirmed using I-specific primers. Plasma ACE activity was determined spectrophotometrically using a commercial kit (Sigma). Plasma t-PA antigen was quantified using 2-site ELISA (Biopool AB).

**Statistics**

Values are reported as mean±SEM. The significance of the relationship between number of D alleles and plasma ACE activity, bradykinin and BK1–5 concentrations, and the BK1–5:bradykinin ratio was determined using bivariate Spearman’s correlation. The ACE genotype effect on bradykinin response was determined by repeated-measures ANOVA in which the withinsubject variable was bradykinin dose and the between-subject variables were ethnicity and ACE I/D genotype. Differences between I/I and D/D groups were determined using Student’s t test or the Mann-Whitney test, with a <0.05 (2-tailed) considered significant.

**Results**

Subject characteristics were similar among the study groups (Table).

<table>
<thead>
<tr>
<th>Variable</th>
<th>ACE I/I (n=9)</th>
<th>ACE I/D (n=9)</th>
<th>ACE D/D (n=9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>31±3</td>
<td>27±2</td>
<td>31±3</td>
<td>0.55</td>
</tr>
<tr>
<td>Race, white: black</td>
<td>5:4</td>
<td>5:4</td>
<td>5:4</td>
<td>1.00</td>
</tr>
<tr>
<td>Sex, male: female</td>
<td>6:3</td>
<td>6:3</td>
<td>5:4</td>
<td>0.85</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.2±1.3</td>
<td>27.1±1.2</td>
<td>25.8±1.4</td>
<td>0.81</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>82.6±2.2</td>
<td>84.5±1.1</td>
<td>87.8±3.0</td>
<td>0.27</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>61.5±3.2</td>
<td>59.0±3.5</td>
<td>60.1±2.0</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Values are mean±SEM or No. of subjects.

Plasma ACE activity increased with number of ACE D alleles (I/I, 20.3±2.3 U/mL; I/D, 25.3±3.3 U/mL; and D/D, 36.8±6.2 U/mL; P=0.017; Figure 1A). During a steady-state infusion of bradykinin (400 ng/min), venous bradykinin concentrations were 726±242 fmol/mL in the I/I group, 469±50 fmol/mL in the I/D group, and 545±104 fmol/mL in the D/D group (P>0.10 for D allele effect; Figure 1B). The presence of the D allele had a significant effect on the venous concentration of the bradykinin metabolite BK1–5 (1113±290, 1520±318, and 1887±388 fmol/mL for the I/I, I/D, and D/D groups, respectively; P=0.027; Figure 1C) and the ratio of BK1–5:bradykinin (1.87±0.35, 3.09±0.40, and 4.31±0.97 for the I/I, I/D, and D/D groups, respectively; P=0.010; Figure 1D). Plasma ACE activity correlated with the BK1–5:bradykinin ratio (r²=0.16, P=0.039).

Blood samples for kinins were not obtained during bradykinin dose escalation, and the study was not designed to measure the concentration-response for bradykinin or BK1–5. However, bradykinin-induced net t-PA release correlated with total kinin (bradykinin+BK1–5) concentration in venous blood. The ratio of BK1–5:bradykinin (1.87±0.35, 3.09±0.40, and 4.31±0.97 for the I/I, I/D, and D/D groups, respectively; P=0.010; Figure 1D) was not affected by ACE I/D genotype (P>0.10). Ethnicity and ACE I/D genotype had an interactive effect on the FBF response to bradykinin (F=4.5, P=0.024), such that vasodilation increased as the number of D alleles increased. ACE I/D genotype effect was most pronounced at submaximal doses (FBF at 100 ng/min: 6.8±1.9, 15.7±5.6, 8.3±0.5, and 14.6±1.6 mL/min per 100 mL of forearm volume for I/I and D/D groups in whites and blacks, respectively; P=0.026 for I/I versus D/D).

**Discussion**

This is the first study to report an association between the ACE D allele and increased bradykinin degradation in humans in vivo. Degradation of bradykinin through the ACE pathway, as measured by the ratio of BK1–5:bradykinin, was greatest in ACE D homozygotes, least in ACE I homozygotes,
and intermediate in heterozygotes. Thus, the number of D alleles had a significant effect on bradykinin metabolism. Bradykinin concentrations tended to be highest in the ACE I/I group, but differences were not statistically significant. Although degradation by ACE seems to be diminished in ACE I homozygotes, bradykinin may be degraded via alternative pathways, such as the aminopeptidase P pathway. Addition-
ally, the extremely short half-life of bradykinin (17 s) and the susceptibility to artifactual production during sampling resulted in a large variance in bradykinin concentrations and could have contributed to a type II error.

One potential limitation of the current study was the use of pharmacological concentrations of bradykinin. However, human sera incubations indicate linear bradykinin degradation through micromolar concentrations. A second potential limitation of the present study was the measurement of BK1–5 concentrations under non-steady-state conditions. BK1–5 has a half-life of 80 to 90 minutes in humans. The prolonged half-life of BK1–5 compared with that of bradykinin accounts for the fact that the ratio of metabolite to parent exceeded unity. However, given the relatively low rate of intra-arterial bradykinin infusion (400 ng/min), most BK1–5 produced before blood sampling would have been diluted in the systemic circulation. On the basis of prior systemic infusion studies, we estimate that the resultant intra-arterial concentration of BK1–5 was <100 fmol/mL. Thus, BK1–5 in forearm venous return during intra-arterial bradykinin infusion represented ongoing local metabolism and not prior production of the metabolite.

In the present study, venous kinin concentration correlated significantly with the net t-PA release across the forearm but not with the vasodilator response to bradykinin. Unlike t-PA release, which reflects a specific effect of bradykinin on the
endothelial cell, vasodilation depends on both endothelial production of nitric oxide and subsequent vascular smooth muscle relaxation, which is influenced by ethnicity. As such, the ethnic heterogeneity and the small sample size of the study groups may have obscured a relationship between kinin concentrations and the vasodilator response to bradykinin. Nevertheless, the correlation between venous kinin concentration and endothelial t-PA release serves to validate the physiological relevance of these measurements.

The functional significance of the association between ACE I/D genotype and bradykinin metabolism depends on the complex interplay between increased degradation of bradykinin and subsequent B2 receptor upregulation secondary to decreased endogenous bradykinin concentrations. One published study suggests that ACE I/D genotype has no effect on the vasodilator response to infused bradykinin. Our preliminary data indicate that the ACE D allele is associated with an increased vasodilator response to exogenous bradykinin, suggesting that enhanced receptor sensitivity predominates over bradykinin degradation. Studies of the impact of ACE I/D polymorphism on the concentration and effects of endogenous kinins are needed to better define the functional significance of this polymorphism as it relates to cardiovascular risk.

In summary, the present study is the first to demonstrate that ACE I/D polymorphism determines the in vivo metabolism of bradykinin in humans. With the development of sensitive assays for measuring endogenous bradykinin and its metabolite, determination of the ratio of BK1–5:bradykinin may constitute a surrogate marker for vascular ACE activity in humans.

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References
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