Bradykinin Type 2 Receptor BE1 Genotype Influences Bradykinin-Dependent Vasodilation During Angiotensin-Converting Enzyme Inhibition

Gary P. Van Guilder, Mias Pretorius, James M. Luther, J. Brian Byrd, Kevin Hill, James V. Gainer, Nancy J. Brown

Abstract—To test the hypothesis that the bradykinin receptor 2 (BDKRB2) BE1+9/−9 polymorphism affects vascular responses to bradykinin, we measured the effect of intra-arterial bradykinin on forearm blood flow and tissue-type plasminogen activator (t-PA) release in 89 normotensive, nonsmoking, white American subjects in whom degradation of bradykinin was blocked by enalaprilat. BE1 genotype frequencies were +9/+9: +9/−9: −9/−9 = 19:42:28. BE1 genotype was associated with systolic blood pressure (121.4±2.8, 113.8±1.8, and 110.6±1.8 mm Hg in +9/+9, +9/−9, and −9/−9 groups, respectively; P=0.007). In the absence of enalaprilat, bradykinin-stimulated forearm blood flow, forearm vascular resistance, and net t-PA release were similar among genotype groups. Enalaprilat increased basal forearm blood flow (P=0.002) and decreased basal forearm vascular resistance (P=0.01) without affecting blood pressure. Enalaprilat enhanced the effect of bradykinin on forearm blood flow, forearm vascular resistance, and t-PA release (all P<0.001). During enalaprilat, forearm blood flow was significantly lower and forearm vascular resistance was higher in response to bradykinin in the +9/+9 compared with +9/−9 and −9/−9 genotype groups (P=0.04 for both). t-PA release tended to be decreased in response to bradykinin in the +9/+9 group (P=0.08). When analyzed separately by gender, BE1 genotype was associated with bradykinin-stimulated t-PA release in angiotensin-converting enzyme inhibitor–treated men but not women (P=0.02 and P=0.77, respectively), after controlling for body mass index. There was no effect of BE1 genotype on responses to the bradykinin type 2 receptor–independent vasodilator methacholine during enalaprilat. In conclusion, the BDKRB2 BE1 polymorphism influences bradykinin type 2 receptor–mediated vasodilation during angiotensin-converting enzyme inhibition. (Hypertension. 2008; 51[part 2]:454-459.)

Key Words: bradykinin • genotype • vasodilation • angiotensin-converting enzyme • plasminogen activators

Angiotensin-converting enzyme (ACE) inhibitors reduce the risk of cardiovascular disease and death attributed to acute atherothrombotic events, including myocardial infarction and stroke.1-3 Beneficial properties of ACE inhibitors on cardiovascular disease include improvements in endothelial function, particularly endothelium-dependent vasodilator and fibrinolytic function.4-8 Studies using bradykinin type 2 (B2) receptor antagonists indicate that endogenous bradykinin contributes to the beneficial effects of ACE inhibition on vasodilation and blood pressure,5,10 as well as on endothelial tissue-type plasminogen activator (t-PA) release.11 The underlying mechanisms by which inhibition of ACE contributes to enhanced bradykinin-stimulated vasodilation and t-PA release have not been completely elucidated but include reduced degradation of endogenous bradykinin and enhanced sensitivity of the B2 receptor.12,13

Given that bradykinin contributes to the salutary effects of ACE inhibitors on vascular function via the B2 receptor, genetic factors that affect B2 receptor sensitivity could impact on responses to ACE inhibitors in vivo. Studies have identified a common variant in the B2 receptor gene (BDKRB2) in which the presence (+9) or absence (−9) of a 9-bp repeat sequence in the noncoding exon 1 (BE1) affects the transcription of the B2 receptor.14 The +9 allele has been associated with decreased B2 receptor gene transcription15 and mRNA expression16 compared with the BE1−9 allele in vitro. Furthermore, the BE1+9/−9 genotype has been associated with significantly higher coronary risk attributable to hypertension as compared with other BE1 genotype groups17 and with reduced regression of left ventricular mass in response to antihypertensive treatment.18 Moreover, Brull et al19 reported an interaction between the ACE I/D and BE1+9/−9 poly-
morphisms on left ventricular growth response to exercise training, such that ventricular hypertrophy was greatest among those with the ACE DD (high ACE activity) and BE1+9/+9 (low receptor expression) genotypes.

We recently reported that the BE1+9/+9 genotype was associated with higher forearm vascular resistance (FVR) in normotensive black Americans and with higher systolic blood pressure (SBP) in normotensive white Americans. BE1 genotype did not influence the vasodilator response to exogenous bradykinin. However, rapid degradation of bradykinin by ACE could have obscured differences in B2 receptor sensitivity among genotype groups. Therefore, we tested the hypothesis that BDKRB2 BE1 genotype affects bradykinin-stimulated vasodilation and t-PA release in the human forearm during ACE inhibition.

Methods

Subjects

Eighty-nine nonobese white American adults (48 males and 41 females) were studied. All of the subjects provided written informed consent. Subjects with a significant cardiovascular, renal, pulmonary, endocrine, or hematologic disease were excluded by history, physical examination, laboratory screening, and ECG. None of the subjects smoked or were taking medications. Subjects with fasting cholesterol >5.7 mmol/L (220 mg/dL) were excluded. Pregnancy was excluded in women of childbearing potential by measurement of urinary β-human chorionic gonadotropin. The protocol was approved by the Vanderbilt University Institutional Review Board and conducted according to the Declaration of Helsinki.

Experimental Protocol

Studies were performed in the morning, in a temperature-controlled room. Subjects were studied in the supine position and in the fasting state. A 20-gauge polyurethane catheter (Cook, Inc) was inserted into the brachial artery of the nondominant arm, and an intravenous catheter was placed in the antecubital vein. Arterial catheter patency was maintained by infusion of 0.9% sodium chloride at a rate of 1 mL/min, and subjects were allowed to rest for 30 minutes before baseline measurements were made and between drug infusions. Heart rate and blood pressure (GE Medical Systems) were continuously monitored throughout the infusion protocol. Forearm blood flow (FBF) was measured using strain-gauge venous occlusion plethysmography (D.E. Hokanson, Bellevue, Wash), as described previously. FBF was measured at baseline and in response to incremental doses of bradykinin (Cinalfa AG, Laufelfingen, Switzerland), methacholine (Pharmaceutical Compounding Center, Nashville, Tenn), and sodium nitroprusside (Gensia Siccor Pharmaceuticals, Irvine, Calif). The 2 latter drugs were used as B2 receptor–independent controls to rule out a B2 receptor–independent effect of bradykinin on vasodilation and t-PA release. Subjects were given bradykinin at 100, 200, and 400 ng/min; methacholine at 3.2, 6.4, and 12.8 μg/min; and sodium nitroprusside at 1.6, 3.2, and 6.4 μg/min. To avoid an order effect, the sequence of drug administration was randomized. Each drug dose was infused for 5 minutes, and FBF was measured during the last 2 minutes of each drug infusion protocol. FBF is presented as milliliters per 100 milliliters of volume of tissue per minute.

After measurement of FBF, arterial and venous samples were collected simultaneously from the experimental arm at baseline and at the end of each drug dose. Plasma samples for measurement of t-PA and plasminogen activator inhibitor (PAI)-1 antigen were collected in tubes containing 10.05 mol/L of acidified sodium citrate and stored at −70°C until the time of assay. Plasma t-PA and PAI-1 antigen concentrations were determined using a 2-site ELISA (Biopool AB). Net endothelial release of t-PA and PAI-1 antigens in response to bradykinin and methacholine was calculated using the following equation:

Net Release = (Ct − Ca) × [FBF × (101 − hematocrit/100)]

where Ct and Ca represent the plasma concentration in the vein and artery, respectively, and forearm plasma flow was calculated from the FBF and hematocrit corrected for 1% trapped plasma. Hematocrit was measured in triplicate using the standard microhematocrit technique and corrected for trapped plasma volume within the trapped erythrocytes. To assess the influence of ACE inhibition on the forearm vascular responses to bradykinin and methacholine among the BE1 genotype groups, a continuous intra-arterial infusion of enalaprilat (Ben Venue Laboratories, Inc) was administered at 0.33 μg/min per 100 mL of forearm volume. Measurement of FBF and net release rates of t-PA antigen during bradykinin and methacholine were repeated in the presence of enalaprilat. To prevent arm swelling, the doses of bradykinin were reduced to 25, 50, and 100 ng/min during enalaprilat infusion.

Genotyping

BE1+9/−9 genotype was determined using PCR amplification, followed by DNA sequencing. The forward 5'-AACGCCCACTGT- TTACATCC-3' and reverse 5'-ACGACCAAGGAAA- ACTTCT-3' primers were designed to encompass the polymorphic promoter region, containing the noncoding exon 1 of the BDKRB2 gene. PCR was performed in a final reaction volume of 25 μL containing 80 ng of genomic DNA, 0.2 μmol/L of each oligonucleotide primer, 100 μmol/L of deoxynucleotide triphosphates, and 0.3 U of TaqDNA polymerase (Roche). The thermocycling procedure (Applied Biosystems) consisted of 35 cycles of 30 seconds at 95°C, 30 seconds at 58°C, and 30 seconds at 72°C, followed by final extension for 5 minutes at 72°C. PCR samples were sequenced at the Vanderbilt DNA Sequencing Facility using BigDye Terminator chemistry and resolved on the ABI 7900 automated sequencer platform (Applied Biosystems).

Statistical Analysis

Differences in baseline characteristics among groups were determined using 1-way ANOVA or Student t test where appropriate. Differences in responses to the vasoactive agents were determined using general linear model repeated-measures ANOVA in which the within-subject variable was dose and the between-subject variables were genotype group, gender, and/or body mass index. When indicated by a significant F value, Scheffe’s test was performed to identify differences between genotype groups. Because we have previously demonstrated that BE1 genotype is associated with SBP in white Americans, FVR was calculated as the ratio of mean arterial pressure:FBF and expressed as arbitrary units (AUs). Data are presented as mean ± SEM. Statistical significance was set at P < 0.05. Statistical analyses were performed using SPSS software version 15.0 (SPSS Inc).

Results

Subject Characteristics

BDKRB2 BE1 genotype distributions were in Hardy-Weinberg equilibrium. Subject characteristics are shown in Table 1. Baseline data and data for bradykinin-stimulated vasodilation in the absence of ACE inhibition (Figure 1) for 78 of the 89 subjects were included in an earlier publication. There were no differences in age, body mass, body mass index (BMI), diastolic blood pressure, resting FBF and FVR, total cholesterol, plasma t-PA, or PAI-1 antigen among genotype groups. SBP was higher in the +9/+9 group compared with the −9/−9 group (P = 0.002) and intermediate in the +9/−9 group (P = 0.02 versus +9/+9). Gender distributions were similar in the −9/−9 and +9/+9 groups; however, women were underrepresented in the +9/+9 genotype group (χ² = 8.95; P = 0.003; 1 degree of freedom).

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**Table 1. Subject Characteristics of BE1 Genotype Groups**

<table>
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<tr>
<th>Variable</th>
<th>−9/−9 (n=28)</th>
<th>+9/−9 (n=42)</th>
<th>+9/+9 (n=19)</th>
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<td>Age, y</td>
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<td>Gender, male/female, n</td>
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<td>Body mass, kg</td>
<td>73.5±2.5</td>
<td>70.6±2.2</td>
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<td>BMI, kg/m²</td>
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<td>Diastolic BP, mm Hg</td>
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<td>FBF, mL/100 mL of tissue per minute</td>
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<td>FVR, AU</td>
<td>22.3±2.0</td>
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<td>t-PA antigen, ng/mL</td>
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<td>PAI-1 antigen, ng/mL</td>
<td>8.8±1.4</td>
<td>11.4±1.0</td>
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Values are mean±SEM unless otherwise specified. BP indicates blood pressure.

*P<0.003 vs +9/−9 and −9/−9 groups.
†P<0.007 for effect of genotype by ANOVA, P=0.02 vs −9/+, and P=0.002 vs −9/−9 genotype groups for posthoc comparison.

**BEI+9/−9 Genotype and Forearm Vascular Response to Bradykinin in the Absence of ACE Inhibition**

Intra-arterial infusion of bradykinin did not affect mean arterial pressure or heart rate. Bradykinin increased FBF and decreased FVR in a dose-dependent manner (Figure 1). FBF and FVR responses to bradykinin were similar in men and women (maximum FBF: 24.9±1.4 mL/100 mL of tissue per minute in men versus 22.4±1.5 mL/100 mL of tissue per minute in women, P=0.24; minimum FVR: 4.0±0.6 AU in men versus 4.9±0.7 AU in women, P=0.22). FBF and FVR responses to bradykinin were also similar among the BE1 genotype groups in the absence of enalaprilat (P=0.88 and P=0.78, respectively).

**BEI+9/+9 Genotype and Forearm Vascular Response to Bradykinin in the Presence of ACE Inhibition**

Intra-arterial enalaprilat increased resting FBF (from 4.1±0.2 to 4.4±0.2 mL/100 mL of tissue per minute; P=0.002) and decreased resting FVR (from 23.6±1.0 to 21.9±0.9 AU; P=0.01) but did not affect mean arterial pressure. As illustrated in Figure 2, enalaprilat potentiated the FBF response to bradykinin to 24.1±0.9 mL/100 mL of tissue per minute during enalaprilat versus 13.8±0.7 mL/100 mL of tissue per minute during vehicle at the 100 ng/min dose (P<0.001). The FVR response to bradykinin was also potentiated by enalaprilat (P<0.001).

During enalaprilat, the forearm vasodilatory response to intra-arterial bradykinin was related to the BE1 genotype. Thus, in the presence of enalaprilat, FBF was significantly lower and FVR higher during bradykinin in the +9/+9 group compared with the +9/−9 and −9/−9 genotype groups combined (P=0.04 for both; Figure 3). During enalaprilat, BMI significantly affected FBF responses to bradykinin (P=0.005). As in the absence of enalaprilat, there was no effect of gender on bradykinin-stimulated FBF, and adjustment for gender and BMI did not alter the significance of the effect of genotype (P=0.03).

**BEI+9/−9 Genotype and Endothelial t-PA Antigen Release**

Basal endothelial t-PA antigen release was similar among the genotype groups (P=0.46). Bradykinin increased t-PA antigen release across the forearm in a dose-dependent manner.

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**Figure 1.** FBF and FVR responses to bradykinin among BDKRB2 BE1 genotype groups in the absence of angiotensin-converting enzyme inhibition. Values are means±SEM.

**Figure 2.** FBF and FVR at baseline and during bradykinin (100 ng/min), in the absence and presence of enalaprilat (0.33 μg/min per 100 mL forearm volume). Values are mean±SEM. *P<0.01 vs baseline vehicle; †P<0.001 vs baseline enalaprilat; ‡P<0.001 vs bradykinin+vehicle.

**Figure 3.** FBF and FVR responses to bradykinin among BDKRB2 BE1 genotype groups in the presence of enalaprilat. Values are mean±SEM. *P<0.05 for BEI+9/+9 vs +9/−9 and −9/−9.
In the absence of enalaprilat, the capacity of the endothelium to release t-PA in response to bradykinin was similar among genotype groups (P=0.64). For example, at the highest dose of bradykinin (400 ng/min), net release rates of t-PA antigen were similar in the −9/−9 (from 0.2±0.8 to 58.8±10.1 ng/100 mL of tissue per minute), +9/−9 (from −0.2±0.5 to 60.8±9.5 ng/100 mL of tissue per minute), and +9/+9 (from 1.3±1.5 to 72.6±9.7 ng/100 mL of tissue per minute) groups.

Intra-arterial administration of enalaprilat increased basal release of t-PA antigen (P<0.001) without affecting basal release of PA1–1 antigen and potentiated the response to exogenous bradykinin (P<0.001; Figure 4). During enalaprilat, the t-PA response to bradykinin tended to be blunted (−55%; P=0.08) in the +9/+9 group (from 5.0±1.7 to 119.1±21.5 ng/100 mL of tissue per minute) compared with the +9/−9 (from 2.0±0.8 to 196.0±29.0 ng/100 mL of tissue per minute) and −9/−9 (from 3.7±1.2 to 165.7±23.0 ng/100 mL of tissue per minute) genotype groups combined. Gender affected bradykinin-stimulated t-PA release in the presence of ACE inhibition (P<0.001). Endothelial t-PA release was 83% higher in women compared with men. After controlling for gender, an effect of BE1 genotype was no longer evident in the overall group. When analyzed separately in men and women, however, BE1 genotype tended to influence the t-PA response to bradykinin during enalaprilat in men such that t-PA release tended to be lower in the +9/+9 group compared with the +9/−9 and −9/−9 genotype groups combined (P=0.05 for dose × BE1 genotype interaction). When BMI was included in the analysis, net t-PA release in response to bradykinin was significantly decreased in +9/+9 men compared with +9/−9 or −9/−9 men (P=0.02 for effect of genotype; Figure 5). In contrast, there was no effect of BE1 genotype on the t-PA response to bradykinin in the presence of ACE inhibition in women, even after controlling for BMI (P=0.77; Figure 5).

**BE1+9/−9 Genotype and Responses to Methacholine and Sodium Nitroprusside**

There was no effect of BE1 genotype on FBF, FVR, or net release of t-PA antigen in response to the endothelium-dependent, B2 receptor-independent agonist methacholine either in the absence or presence of enalaprilat (Table 2). In addition, there were no significant differences among the −9/−9 (from 4.1±0.3 to 20.9±1.5 mL/100 mL of tissue per minute), +9/−9 (from 4.4±0.3 to 19.6±1.1 mL/100 mL of tissue per minute), and +9/+9 (from 4.4±0.3 to 19.0±1.4 mL/100 mL of tissue per minute) genotype groups in the forearm vasodilator responses to sodium nitroprusside.

**Discussion**

We have reported previously that the BDKRB2 BE1 polymorphism associates with SBP in normotensive white Amer-
Table 2. Forearm Vascular Responses to Methacholine in the Absence and Presence of ACE Inhibition Among the BE1 Genotype Groups

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Values are mean±SEM.

*P<0.05 vs baseline.

ics but does not affect the vasodilator response to exogenous bradykinin in the forearm.20 Here we report the effect of intra-arterial bradykinin infusion during administration of the ACE inhibitor enalapril. As expected,1,13,24 ACE inhibition potentiated the effect of bradykinin on FBF and t-PA release. More importantly, BEKR2 BE1 genotype influenced bradykinin-stimulated vasodilation and tended to influence t-PA release during ACE inhibition, such that individuals homozygous for the BE1+9 allele exhibited blunted responses to bradykinin compared with carriers of a −9 allele. To our knowledge, this study is the first to address the effect of variation at the BEKR2 gene on the vascular effects of bradykinin during ACE inhibition in humans. Moreover, there was no effect of BEKR2 BE1 genotype on nitroprusside-stimulated vasodilation or methacholine-stimulated vasodilation or t-PA release, either in the absence or presence of ACE inhibition, suggesting that the BE1 genotype specifically affected B2 receptor sensitivity.

We have reported previously that, during ACE inhibition, bradykinin-stimulated t-PA release is enhanced in women compared with men, whereas there is no effect of gender on bradykinin-stimulated vasodilation.11 In the current study we also observed an interactive effect of the BDKRB2 BE1+9/−9 polymorphism and gender on bradykinin-mediated t-PA release, such that the BE1+9/9+9 genotype was associated with decreased t-PA release in men but not in women. The mechanism of this gender effect requires further exploration. One nonrandomized study of hormone therapy suggests that estrogen affects bradykinin-stimulated t-PA release;24 however, endogenous and bradykinin-stimulated t-PA release are also increased in ACE inhibitor–treated postmenopausal women compared with age-matched men, suggesting an estrogen-independent effect of gender.23 In this regard, decreased bradykinin-stimulated t-PA release in men during ACE inhibition may reflect an effect of testosterone to decrease bradykinin-stimulated calcium influx.26

Two potential limitations merit discussion. First, in an effort to minimize variability in the present study, we included white, healthy, nonsmoking adults of similar age who were not taking medications. We have reported previously that ethnicity influences the effect of the BDKRB2 BE1 genotype on resting vascular function, such that the BE1+9/9+9 genotype is associated with increased SBP in normotensive white Americans and with increased FVR in normotensive black Americans.20 In addition, ethnicity has been shown to influence the cardioprotective effects of ACE inhibitors.25 For this reason, the findings of the present study are not necessarily generalizable to other ethnic groups.

In addition, by chance, men were overrepresented in the BE1+9/9+9 genotype group. Because there was no effect of gender on the vasodilator response to bradykinin, either in the presence or absence of enalapril, this is unlikely to have impacted the relationship between BDKRB2 BE1 genotype and the vasodilator response to bradykinin during ACE inhibition. Indeed, adjusting for gender did not alter the results. Gender did, however, influence bradykinin-stimulated t-PA release. We, therefore, conducted separate analyses of the effect of BE1 genotype on bradykinin-stimulated t-PA release in men and women. The observation that BE1 genotype did not affect bradykinin-stimulated t-PA release in women but that t-PA release was 2-fold greater in women compared with men, regardless of genotype, suggests that increased endothelial storage of t-PA trumps genetic variability at the B2 receptor in determining bradykinin-stimulated t-PA release in women.

**Perspectives**

ACE inhibitors reduce the risk of cardiovascular disease and death attributed to acute atherothrombotic events, including myocardial infarction and stroke.1–3 Bradykinin contributes to vasodilation and endogenous t-PA release during ACE inhibition via its B2 receptor.11 This study provides the first evidence that the BE1+9/9−9 polymorphism influences bradykinin-mediated vasodilation, as well as bradykinin-mediated t-PA release in men, during ACE inhibition. Preventing the degradation of bradykinin by ACE unmasked the association between the bradykinin B2 receptor BE1 genotype and responses to bradykinin.

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