Components of the kallikrein kinin system have been associated with the pathophysiology of hypertension in animal and human studies. In this study, we examined the distribution of four different polymorphisms of the kinin B₁ and B₂ receptor genes in a population of 120 normotensive and 77 hypertensive African-Americans. Allelic frequencies for three of the four polymorphisms were significantly different from those previously reported in Caucasian populations. Among the polymorphisms analyzed, a potentially functionally significant polymorphism in the core promoter of the kinin B₂ receptor (C⁻⁵⁸→T transition) displayed an increased prevalence of the C⁻⁵⁸ allele in the hypertensive patients as compared with the controls (0.75 v 0.62, P = .009). Thus, this B₂ receptor promoter polymorphism may represent a susceptibility marker for essential hypertension in African-Americans. Am J Hypertens 2000;13:1268–1273 © 2000 American Journal of Hypertension, Ltd.

**KEY WORDS:** Hypertension, Allelic polymorphism, African-Americans, kinin B₂ receptor.

Hypertension is a complex multifactorial disorder with genetic heritability averaging approximately 30%. The search for genetic determinants has focused primarily on genes involved in the renal control of sodium balance. Rare genetic mutations in the renal epithelial sodium channel gene and a chimeric gene duplication involving 11 β-hydroxylase and aldosterone synthase have been described, but affect only a small portion of hypertensives. Common susceptibility genes that confer an increased risk for developing hypertension have proved more elusive. With regard to the renin-angiotensin system, numerous studies support an association of variants of the angiotensinogen gene with hypertension, whereas studies of other renin-angiotensin genetic targets so far have been inconsistent.

Components of the kallikrein-kinin system (KKS) play important roles in blood pressure (BP) and renal sodium regulation. Two subtypes (B₁ and B₂) of kinin receptors (R), predominantly the B₂R, mediate the known effects of the KKS. The B₂R displays a protective role in the development of hypertension, renal, and cardiovascular pathology. We and others have characterized a number of polymorphic sites for both receptor genes, some of which have been
associated with clinically significant inflammation and renal complications. In addition, in vitro studies suggest that polymorphisms in the B2R promoter are associated with decreased transcriptional activity.

Complicating the search for susceptibility genes is the difference in prevalence and expression of hypertension among various ethnic groups. Essential hypertension is at least twice as common among African-Americans as in the general population, and the prevalence of salt sensitivity in hypertensive African-Americans has been reported to be nearly twice that of hypertensive Caucasians. The aim of the present study was to determine the allelic frequency of these significant B1 and B2 receptor polymorphisms in hypertensive and normotensive African-Americans.

METHODS

Subjects The study protocol was approved by the Vanderbilt Institutional Review Board, and subjects gave written informed consent. High molecular weight DNA was extracted from peripheral blood leukocytes obtained from 197 African-Americans (120 unrelated healthy volunteers and 77 patients with essential hypertension from the Vanderbilt Hypertension Clinic). Ethnicity was self-reported. Subjects were defined as normotensive if they had a seated systolic blood pressure < 140 mm Hg, diastolic blood pressure (DBP) < 90 mm Hg, and no history of identification or treatment of hypertension. Hypertensive subjects in this study had either established hypertension (defined by chronic antihypertensive medication treatment) or a seated DBP > 90 mm Hg on at least three occasions. Secondary hypertension was excluded by history and physical examination. Fifth-phase Korotkoff sounds were used for DBP measurements. Family history of hypertension was self-reported and confined to first-degree relatives.

Detection of Bradykinin Receptor Polymorphisms The frequencies of two B2R and two B1R polymorphisms were examined. The first B2R polymorphism was a C<sup>181</sup> → T transition in exon 2, which leads to an arginine to cysteine substitution at position 14 in the B2R receptor protein. This polymorphism was detected using polymerase chain reaction (PCR) primers and conditions as indicated in previous reports. A second B2R polymorphism (C<sup>58</sup> → T transition), located in the promoter region, was also detected using previously published methods.

The two B1R polymorphisms examined are both single base substitutions (A<sup>1098</sup> → G in a noncoding part of exon 3 and a G<sup>699</sup> → C transition in the B1R promoter). They were detected using restriction fragment length polymorphisms (RFLP) of the corresponding PCR fragments after digestion with the restriction endonucleases TaqI and AciI, respectively.

Statistical Analysis Baseline characteristics between normotensive and hypertensive subjects were compared using t tests or χ² analysis where appropriate. The genotype frequencies for each polymorphism were tested for deviation from Hardy-Weinberg equilibrium by χ² goodness-of-fit analysis with 1 df. Genotype distributions between normotensive and hypertensive subjects were compared using a χ² test with 2 df. Adjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated using logistic regression with multivariate analysis. All statistical tests were two-sided, and a value of P < .05 was considered statistically significant.

RESULTS

The genotype variations of four kinin polymorphisms (two B2R and two B1R) were examined in a population of normotensive and hypertensive African-Americans from the Tennessee region. Baseline subject characteristics of the population studied are presented in Table 1. Compared with the normotensive subjects, hypertensive subjects were older, heavier, and had a stronger family history of hypertension. Mean arterial pressure was significantly higher in the hypertensive subjects than the normotensive subjects. Gender distribution was similar between groups.

Genotype distributions were in accordance with Hardy-Weinberg equilibrium (data not shown), and were similar between hypertensive and normotensive subjects for each polymorphism studied except for the B2R promoter polymorphism, C<sup>58</sup> / T (P = .009), as shown in Table 2. The C<sup>58</sup> allele frequency was significantly greater in hypertensive compared with normotensive African-Americans (0.75 v 0.62, χ² = 7.9, P = .005). Using multivariate analysis, the B2R C<sup>58</sup> / T polymorphism was significantly associated with hypertension (P = .03) after adjusting for age, weight, and family history of hypertension. In addition, there was no statistical difference in age or weight among...
B2R C\textsuperscript{−58}/T genotypes within either hypertensive or normotensive groups (data not shown). Odds ratios unadjusted and adjusted for age, weight, and family history of hypertension are presented in Table 2. Compared with the reference TT\textsuperscript{−58} genotype, the adjusted OR of having hypertension in those subjects carrying the CT and CC genotypes were 10.5 (95\% CI, 1.2 to 96.1) and 9.5 (CI, 1.1 to 85.4), respectively.

The prevalence of the B2R C\textsuperscript{−58} allele in normotensive African-Americans was similar to that reported in Caucasians.\textsuperscript{23} However, as shown in Table 3, the prevalence of the rare allele for the other three polymorphisms was significantly lower in the African-American population (B2R T\textsuperscript{181} allele, \(P < .001\); B2R G\textsuperscript{1098} allele, \(P = .003\); B1R C\textsuperscript{−699} allele, \(P < .001\)) as compared with frequencies that we and others have reported in Caucasian populations.\textsuperscript{22,25}

**DISCUSSION**

Since the finding in 1934 that urinary kallikrein was decreased in subjects with essential hypertension compared with those with normal BP,\textsuperscript{29} it has been thought that components of the KKS could contribute to the pathophysiology of hypertension. More recently, experimental data have shown a protective role of B2Rs in the development of hypertension and cardiovascular pathology.\textsuperscript{30–32} The B2Rs mediate the majority of in vivo effects attributed to kinins under normal physiologic conditions: vasodilation, pain, increased vascular permeability, and increased production of eicosanoids and nitric oxide.\textsuperscript{33,34} A confirmation of KKS involvement in hypertension has been shown in recent studies using the B2R-knockout mouse model.\textsuperscript{35} Compared with a control strain, B2R-knockouts have a moderately increased basal BP and, upon exposure to an excess of dietary NaCl, exhibit severe hypertension with end organ damage.\textsuperscript{36–38} These studies emphasize the importance of bradykinin in the development of salt-sensitive hypertension.

In humans, the prevalence of salt-sensitive hypertension has been reported to be greater in African-Americans compared with Caucasians.\textsuperscript{28} Several hormonal determinants including decreased KKS activity have been proposed to account for salt sensitivity of BP.\textsuperscript{28} Additionally, KKS components have been shown to contribute to the therapeutic effects of angiotensin converting enzyme inhibitors,\textsuperscript{39–45} antihypertensive medications observed to be less efficacious in African-Americans than in Caucasians.\textsuperscript{36}

Kammerer et al have recently reported the organi-
zation and regulatory region of the human B2R and subsequently identified four polymorphisms including a promoter polymorphism 58 bases upstream to the transcript initiation site (C-58/T). In vitro experiments suggest that the C-58/T B2R promoter polymorphism may influence transcriptional activity. Braun et al observed decreased reporter expression in human embryonic kidney cells transfected with the C-58 allele and exon 1 compared with the those transfected with the T-58 allele. Mukae et al recently reported an increased frequency of the C-58 allele in hypertensive Japanese subjects compared with normotensives.

In the present study, we compared the allelic frequencies of two B2R and two B1R polymorphisms among African-American normotensive and hypertensive individuals. Among these, the frequency of the C-58 allele of the B2R promoter was significantly increased in the hypertensive patients in comparison with the controls (Table 2). The association of the C-58 allele with hypertension was independent of the effects of age, weight, and family history of hypertension. Thus, the B2R C-58 allele may have functional relevance, or is in linkage disequilibrium, with a functional variant of the B2R that predisposes its carriers to hypertension.

The three other allelic polymorphisms of the kinin receptor genes were found to be clinically neutral among normotensive and hypertensive African-Americans. The findings of decreased B2R transcriptional activity associated with the C-58 allele and an increased prevalence of that allele in hypertensives compared with a normotensive population, as shown in this study, suggest a pathophysiologic mechanism by which the B2R contributes to the development of hypertension.

The frequency of the B2R C-58 allele in African-Americans was similar to that previously reported in Caucasian populations. However, for the other three kinin receptor polymorphisms, we found a significantly lower frequency of the rare allele in African-Americans compared with frequencies that we and others have reported in Caucasians. Race-related differences have been reported for another kinin receptor polymorphism, a B2R exon 1 insertion (+)/deletion (−) polymorphism in which Lung et al found a significant over-representation of the (−) allele in Asians compared with either Caucasians or African-Americans. Additionally, Lung et al found an association of the B2R (−) allele with symptomatic cases of hereditary angioedema with C1 inhibitor deficiency. However, the significance of racial differences in these kinin polymorphisms is not known.

The available evidence indicates that in most species, B1Rs are probably more involved in immunopathology than in hypertension and cardiovascular pathology. The B2R promoter polymorphism has been suggested as a nonetiologic marker of symptomatic inflammatory bowel disease. However, we have recently described an association of end-stage renal disease with altered frequencies of the B1R promoter polymorphism as well as the B2R exon 2 polymorphism. Because of the proximity of the B1 and B2 kinin receptors on chromosome 14q32, inclusion of B2R polymorphisms improves the likelihood of detecting a functionally significant mutation at or near the 14q32 chromosomal locus.

Additional studies are required to confirm the association of the C-58 allele with essential hypertension. Compared with the hypertensives in our study, the normotensive group was younger and, therefore, may have included subjects who would later develop hypertension. The inclusion of such subjects, however, would have resulted in a decreased potential to detect an association of B2R variants with hypertension.

In summary, we report an association of a functionally significant B2R promoter polymorphism with essential hypertension in an African-American population. These findings support a pathophysiologic role of the KKS in BP regulation and the B2R C-58 allele as a potential susceptibility marker for the development of hypertension.

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