**β₂-Adrenoceptor Polymorphism Determines Vascular Reactivity in Humans**

John R. Cockcroft, Anastasios G. Gazis, Debbie J. Cross, Amanda Wheatley, Jane Dewar, Ian P. Hall, Joseph P. Noon

*Abstract*—Altered β-adrenergic regulation has been reported in individuals with hypertension. The variability in vascular responsiveness to β-agonists, such as isoproterenol, observed in humans may be explained partially by β₂-adrenoceptor polymorphism. Individuals with the Gln27 form of the receptor may show reduced vascular reactivity because of downregulation expression of the receptor in the vasculature. We screened 127 normotensive white subjects, 37 of whom were homozygous for these alleles. Thirty-two subjects (17 Gln27 and 15 Glu27) agreed to receive brachial artery infusions of isoproterenol at doses of 1 to 300 ng·min⁻¹; forearm blood flow was measured by using venous occlusion plethysmography. Of these subjects, 25 (12 Glu27 and 13 Gln27) received local doses of isoproterenol (0.3 to 30.0 ng·min⁻¹) via a dorsal hand vein preconstricted with norepinephrine. Compared with subjects homozygous for the Glu27 allele, subjects with the Gln27 substitution had lower baseline blood flow and, in response to isoproterenol, had a significantly attenuated increase in forearm blood flow. This pattern was more marked in veins. We also studied the relationship between the position 16 polymorphism and vascular reactivity. Homozygotes for Arg16 had significantly lower basal blood flow and attenuated increases in forearm blood flow compared with the Gly16 homozygotes. This was significant in veins but not in arteries. Thus, β₂-adrenoceptor genotype determines vascular responses to isoproterenol in forearm resistance vessels and in capacitance vessels. Further studies are necessary to establish whether β₂-adrenoceptor polymorphisms are important in the genesis of hypertension. (*Hypertension*. 2000;36:371-375.)

**Key Words:** receptors, adrenergic, beta ■ blood flow ■ veins ■ isoproterenol ■ genes

The effects of β₂-agonists are produced at the cell level by binding to the β₂-adrenoceptor. The vascular endothelium and the vascular smooth muscle express β₂-adrenoceptors, and the infusion of isoproterenol into the forearm vascular bed¹ and into dorsal hand veins²,³ of normotensive and hypertensive individuals produces a vasodilator response. Part of this response is endothelium dependent,⁴,⁵ because of the release of NO oxide from the vascular endothelium.

After stimulation with agonists, β₂-adrenoceptors in the vascular bed rapidly downregulate.⁶ Previous work by our group and others² in transformed cell lines expressing the different polymorphic forms of the β₂-adrenoceptor and in primary human airway smooth muscle cells of known β₂-adrenoceptor genotype has shown that downregulation is determined in part by β₂-adrenoceptor polymorphism. Four amino acid polymorphisms have been reported within the β₂-adrenoceptor gene; all are due to single base substitutions, although only 2 of these polymorphisms are common in the general population and functionally important. The Arg/Gly16 polymorphism results in increased downregulation when assessed by receptor binding and also by adenyl cyclase assays in transfected cell systems but produces relatively less marked effects in primary human airway smooth muscle cells.⁷ In contrast, the Gln/Glu27 polymorphism has marked effects in the transfected cell system and in human smooth muscle cells in culture, with the Glu27 form of the receptor downregulating to a much greater extent than the “wild type” (Gln27). In view of these observations, we hypothesized that the vascular response to infused isoproterenol might partly be dependent on β₂-adrenoceptor genotype.

**Methods**

**Study Design**

We used a prospective double-blind study design. Young, male, normotensive, white volunteers were recruited from the local community. Subject characteristics are listed in Tables 1 and 2. We studied groups of individuals homozygous for either Gln27 or Glu27 alleles. One hundred twenty-seven subjects were screened, of whom 37 were homozygous for these alleles; 32 agreed to participate in arterial studies (15 Gln27 and 17 Glu27), and 25 agreed to participate in vein studies (12 Glu27 and 13 Gln27). Subjects attended the clinical laboratory, and forearm blood flow and vein studies were conducted on separate occasions. Neither the investigator conducting each study nor the subject was aware of the subject’s genotype. All forearm blood flow and hand vein traces were analyzed in a blinded

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Genotyping

β2-Adrenoceptor genotype was determined by allele-specific oligonucleotide hybridization as previously described. In brief, genomic DNA was extracted from a 5-mL sample of whole blood in EDTA by use of a commercially available kit (Nucleon, ScotLab). A 234-bp DNA was extracted from a 5-mL sample of whole blood in EDTA by use of a commercially available kit (Nucleon, ScotLab). A 234-bp DNA was extracted from a 5-mL sample of whole blood in EDTA by use of a commercially available kit (Nucleon, ScotLab). A 234-bp DNA was extracted from a 5-mL sample of whole blood in EDTA by use of a commercially available kit (Nucleon, ScotLab). A 234-bp

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</table>

Values are mean±SEM.

Genotyping

β2-Adrenoceptor genotype was determined by allele-specific oligonucleotide hybridization as previously described. In brief, genomic DNA was extracted from a 5-mL sample of whole blood in EDTA by use of a commercially available kit (Nucleon, ScotLab). A 234-bp fragment spanning the polymorphisms of interest from the 5 prime end of the β2-adrenoceptor was generated by polymerase chain reaction (PCR). The 50-μL PCR reaction contained 1 μL of genomic DNA, 34 μL of water, 200 μmol/L of each dNTP, 5 μL of PCR (10× buffer), and 1.5 mmol/L of MgCl2; 2 μmol/L of each primer and 1 unit of Taq polymerase was added per reaction. The primer sequences used were upstream CCC AGC CAG TGC GCT TACCT and downstream CCG TCT GCA GAG CGAAC. The reaction consisted of 36 cycles (melting temperature 94°C, 90 s; annealing temperature 60°C, 90 s; extension temperature 72°C, 90 s) with an initial period of 5 minutes at 94°C during the first cycle and a 10-minute extension at 72°C after the last cycle. PCR product (1 μL) was then applied to duplicate Hybond N+ filters by use of a dot-blot apparatus, and genotype was finally determined by allele-specific oligonucleotide hybridization. The probes used for hybridization were Gln27 (CAC GCA GGA AAG GGA CGAG), Glu27 (CACGCA GCA AAG GGA CGAG), Arg16 (GCA CCC AAT AGA AGC CATG), and Gly16 (GCA CCC AAT AGA AGC CATG). A 10-fold excess of cold probe was used in the initial part of the hybridization for the Arg/Gly polymorphism, and a 30-fold excess of cold probe was used for the Gln/Glu27 polymorphism. Probe filters were exposed to x-ray film overnight, and genotype was determined. Direct sequencing of a random selection of samples was performed to ensure accuracy.

Arterial Studies

Subjects attended a quiet and temperature-controlled (23±2°C) clinical laboratory. Blood was taken for serum cholesterol measurement, and blood pressure was measured in triplicate by using a Dinamap automatic vital signs monitor after the subject underwent 15 minutes of supine rest and was again seated. Forearm blood flow was measured in both arms with the use of venous occlusion plethysmography with temperature-compensated electrically calibrated strain gauges as previously described. A 27-gauge mounted steel needle was inserted into the left brachial artery under local anesthesia (1% lidocaine hydrochloride). Saline or isoproterenol was then infused at a rate of 1.0 mL·min⁻¹ by means of a constant rate infusion pump. Basal blood flow was recorded after 12 minutes of saline infusion. Isoproterenol was then infused intra-arterially at doses of 1, 3, 10, 30, 100, and 300 ng·min⁻¹; each dose was infused at 1.0 mL·min⁻¹ for 6 minutes. Forearm blood flow was measured over the last 3 minutes of each infusion period, and the mean of the final 5 measurements was used for analysis.

Dorsal Hand Vein Studies

Subjects (Tables 1 and 2) were sent to a temperature-controlled (25±1°C) clinical laboratory. With the subjects at rest in this warm atmosphere, their veins had no intrinsic tone; thus, their veins were preconstricted with norepinephrine (1 to 128 ng·min⁻¹) until 70% to 80% constriction was achieved. Isoproterenol was then administered
at doses of 0.3, 1.0, 3.0, 10.0, and 30.0 ng · min⁻¹. Each dose was given for 10 minutes by use of the Aellig linear displacement technique, and the vein diameter was measured as an index of dilatation during the final 5 minutes. Several measurements were taken during this 5-minute period, but the mean of the final 4 measurements was used in the analysis.

**Statistical Analysis**

Blood flow data are presented as mean ± SEM. Basal blood flow and area under the curves were compared by unpaired *t* tests. For vein studies, venodilation was expressed as percent reversal of norepinephrine-induced constriction and analyzed by repeated-measures ANOVA. Where ANOVA showed a significant treatment effect (*P*<0.05), effects due to genotype were compared at each dose by Student paired *t* test. Probability values were corrected for the total number of comparisons by the Bonferroni method.

**Results**

**Arterial Studies**

Blood flow in the noninfused arm did not change significantly throughout the study period in any of the subjects, confirming that at the doses used, isoproterenol did not have any systemic effects. Basal blood flow was lower in subjects with the Gln27 allele than in subjects with the Glu27 allele (3.21±0.34 and 4.43±0.64 mL · 100 mL⁻¹ · min⁻¹, respectively), although this difference did not quite reach statistical significance (*P* = 0.05). An explanation for this observation is that there is chronic receptor downregulation in the Gln27 homozygotes. In the Glu27 homozygotes, blood flow in the infused arm increased from 4.43±0.64 to 19.41±2.32 mL · 100 mL⁻¹ · min⁻¹ at the highest dose of isoproterenol (300 ng · min⁻¹) (Figure 1). Blood flow responses to isoproterenol in the Gln27 homozygotes were significantly attenuated, increasing from 3.21±0.34 to 13.95±1.53 mL · 100 mL⁻¹ · min⁻¹ (*P*<0.05). This effect was apparent at all concentrations of isoproterenol studied (*P* = 0.03 for area under curve), suggesting that the difference in vascular reactivity to isoproterenol is related to the baseline shift in the Glu27 homozygotes.

Preliminary data have suggested that the amino acid 16 polymorphism may be linked to hypertension. Because the subjects for the present study were defined by the amino acid 27 polymorphism, we also had individuals heterozygous at amino acid 16. Therefore, we also analyzed the blood flow responses to isoproterenol in terms of the Arg/Gly16 polymorphism (18 homozygotes [7 Arg16 and 11 Gly16], 9 heterozygotes, and 5 not determined). Subject characteristics are listed in Table 2. Basal blood flow was lower in subjects homozygous for Arg16 than in subjects homozygous for Gly16 (2.48±0.51 and 4.85±0.81 mL · 100 mL⁻¹ · min⁻¹, respectively; *P* = <0.02). Basal flow (3.19±0.53 mL · 100 mL⁻¹ · min⁻¹) in the heterozygotes was intermediate and significantly different from that in the Gly16 homozygotes (*P*<0.05). In subjects homozygous for the Arg16 allele, blood flow increased from 2.48±0.51 to 12.97±2.32 mL · 100 mL⁻¹ · min⁻¹ at the highest dose of isoproterenol (Figure 2). Increase in blood flow in the Gly16 homozygotes was greater (from 4.85±0.81 to 19.94±3.48 mL · 100 mL⁻¹ · min⁻¹), although this difference was not significant. Although blood flow in the heterozygotes increased to a degree similar to that in the Arg16 homozygotes (from 3.19±0.53 to 12.92±2.24 mL · 100 mL⁻¹ · min⁻¹), this group did not differ significantly from either the Gly16 or Arg16 homozygotes.

These initially surprising observations are probably explained by the strong linkage disequilibrium that exists between the amino acid 16 and 27 polymorphisms in the white population. Of our subjects who were homozygous for the Gln27 allele, only 1 was also homozygous for Gly16, with 7 being homozygous for Arg16 and the rest being heterozygotes (n=9). Further studies will be required to examine the potential interactions between these 2 polymorphisms.

**Dorsal Hand Vein Studies**

The dose of norepinephrine required to achieve 78% constriction (from basal levels of dilatation) was lower in subjects homozygous for the Glu27 polymorphism (ED₉₀ 6 ng · min⁻¹) compared with the Gln27 subjects (ED₉₀ 12 ng · min⁻¹). Dorsal hand vein dilator in response to isoproterenol was markedly and significantly attenuated in Glu27 compared with Gln27 subjects (ANOVA, *P*<0.001) (Figure 3). Arg16 homozygotes and Arg/Gly16 heterozygotes showed similar responses to isoproterenol, but responses in Gly16 homozygotes were greater by 3-fold at higher doses (Figure 4).

**Discussion**

We have demonstrated a relationship between the Gln/Glu27 β₂-adrenoceptor polymorphism and forearm vascular reactiv-
disequilibrium with the known differences in the distribution of the South African populations than in whites; hence, racial observed recently that the Glu27 allele is much rarer in black or near to the RFLP is due to a degenerate polymorphism at base 523 in the
effect of genotype in vascular smooth muscle cells in
phisms at codons 16 and 27. To date, there are no reports on
to isoproterenol in a group of male normotensive subjects. Support for these findings comes from 2 observations. First, there is marked variability in the vasodilator response to infused isoproterenol between individuals. In particular, isoproterenol-mediated vasodilation is attenuated in black normotensive subjects and in black normotensive subjects from families with a history of hypertension. We have observed recently that the Glu27 allele is much rarer in black South African populations than in whites; hence, racial differences in the distribution of the β2-adrenoceptor polymorphism could explain altered vascular reactivity. Second, a recent study has shown that a restriction fragment length polymorphism (RFLP) related to the β2-adrenoceptor locus on chromosome 5q31 is associated with hypertension in blacks. This suggests that in defined populations, a locus in or near to the β2-adrenoceptor may be important for the development of hypertension. We have observed that this RFLP is due to a degenerate polymorphism at base 523 in the β2-adrenoceptor gene and that this RFLP is in linkage disequilibrium with the known β2-adrenoceptor polymorphisms at codons 16 and 27. To date, there are no reports on the effect of genotype in vascular smooth muscle cells in culture, although reduced expression of the β2-adrenoceptor has been noted on cultured fibroblasts from young normoten-
sive salt-sensitive subjects compared with salt-resistant subjects.

The vasodilator response to isoproterenol in the human forearm vascular bed recently has been shown to be endothelium dependent and can be significantly blunted by the NO synthase inhibitor N'pi-monomethyl-L-arginine. Endothelium-dependent vasodilation can be attenuated by conditions such as hypercholesterolemia, diabetes mellitus, and smoking and, therefore, could be a potential explanation for the decreased response in the Gln27 homozygotes. However, this is unlikely to be the case in the present study because none of the subjects were diabetic, and the Gln27/Glu27 homozygotes were well matched in terms of age, blood pressure, cholesterol, and smoking status (Table 1). A physiological role for β2-adrenoceptor polymorphisms also has been shown in studies on airway responsiveness. Asthmatic subjects homozygous for the Glu27 genotype have less reactive airways than the Gln27 homozygotes, suggesting that the β2-adrenoceptor polymorphism may be important in determining airway responses. In the present study, we found that compared with a group of individuals homozygous for Gln27, individuals homozygous for the Glu27 β2-adrenoceptor polymorphism have an increased vasodilator response to infused isoproterenol. The fact that subjects with the Gln27 genotype also had lower baseline flow suggests that such individuals may be chronically downregulated in terms of their response to β2-agonists. Taken together, these data imply that responses to β2-agonists in a range of different tissues and cell types in an individual may be partly determined by β2-adrenoceptor polymorphic status.

The increased vasodilator responses to isoproterenol in Glu27 compared with Gln27 homozygotes were more pronounced in veins than in arteries. Baseline flows in veins were similar in all genotypes because veins were preconstricted with norepinephrine by 80% in all subjects. However, an interesting finding was that Gln27 homozygotes required twice the concentration of norepinephrine than that used for Glu27 subjects to achieve similar levels of constriction. These differences in norepinephrine sensitivity require further investigation.

Our potential difficulty in interpreting data from the present study is the strong linkage disequilibrium that exists between the codon 16 and 27 β2-adrenoceptor polymorphisms. In vitro data suggest that the effects of the codon 16 polymorphism predominate over those at codon 27, although this requires confirmation. Dissecting the effects of given combinations of alleles will require studies in populations with different allelic frequencies: in particular, it would be interesting to study vascular responsiveness in the black South African population, in which the Glu27 allele is much rarer. In summary, we have demonstrated a relationship between the Gln/Glu27 β2-adrenoceptor polymorphism and forearm vascular reactivity to isoproterenol in a group of male normotensive subjects. Ideally, we would like to have studied a group of black individuals homozygous for the Glu27 polymorphism to confirm that the reduced responsiveness seen in black populations is related to altered distribution of β2-adrenoceptor polymorphisms. However, given the low
prevalence (~5%) of these individuals, this will prove logistically difficult to study.

Given that the β2-adrenoceptor polymorphism appears to contribute to determining vascular reactivity, the potential contribution of these polymorphisms to determining disease severity or response to therapy in hypertensive individuals requires further study.

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References


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