ß-2 Adrenergic Receptor Variants Affect Resting Blood Pressure and Agonist-Induced Vasodilation in Young Adult Caucasians

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Hypertension 1999;33;1425-1430
Hypertension is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0194-911X. Online ISSN: 1524-4563

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Abstract—Recent evidence suggests that the prodownregulatory Gly16 allele of the β-2 adrenergic receptor (β-2 AR) is associated with essential hypertension in African Caribbeans. To further investigate the effect of the glycine (Gly)16 and arginine (Arg)16 β-2 AR variants on hemodynamics, we investigated the agonist-mediated in vivo vasodilation in normotensive Austrian Caucasians and analyzed the results with respect to the Gly16/Arg16 polymorphism. Fifty-seven normotensive men, 20 to 32 years of age with body mass index of 18.7 to 29.9 kg/m^2, were genotyped for the Arg16/Gly16 β-2 AR alleles. All 15 Gly16/Gly16 subjects, all 12 Arg16/Arg/16 subjects, and 27 of 30 heterozygous subjects underwent hemodynamic measurements while supine after an overnight fast. The observers were unaware of the subjects' genotypes. The subjects received a graded infusion of the selective β-2 AR agonist salbutamol (0.07, 0.14, and 0.21 μg/kg per minute, respectively), each dose over 8 minutes. Stroke volume and blood pressure were determined continuously by means of impedance cardiography and oscillometry, respectively. The last 4 minutes of each infusion were evaluated statistically. Basal mean blood pressure was higher in the Gly16/Gly16 subjects compared with Arg16/Arg16 subjects (mean±SD: 81.6±6.14 versus 75.2±4.93 mm Hg, $P<0.01$). Homozygous Gly16 subjects showed a significantly decreased vasodilation during the first dose of salbutamol infusion compared with Arg16/Arg16 subjects (Δtotal peripheral resistance index −17.9±14.4 versus −30.6±8.3%, $P<0.01$) despite increased sympathetic counterregulation in the Arg16/Arg16 group (Δheart rate +16.9±7.0% versus +8.6±7.0%, $P<0.01$; Δcardiac index +39.5±18.5% versus 21.4±18.8%, $P<0.05$). Our results provide additional evidence that the Gly16/Arg16 alleles of the β-2 AR are intimately related to blood pressure regulation and deserve further studies in the pathogenesis of essential hypertension. **(*Hypertension*. 1999;33:1425-1430.)**

Key Words: hypertension, essential ■ molecular genetics ■ β-2 adrenergic receptor ■ Arg16/Gly16 allele ■ vasodilation ■ impedance cardiography

The β-2 adrenergic receptor (β-2 AR) has been implicated in the pathogenesis of hypertension in studies suggesting defective β-2–mediated vasodilation.[1] Blunted vasodilation in response to β-2 AR stimulation has been reported both in white patients with hypertension[2–4] and in normotensive African Americans.[5] The reasons for this blunted vasodilatory response after exposure to β-2 AR agonists are unclear. Interestingly, the blunted vasodilation can be corrected by a low sodium diet, a condition that may promote upregulation of β-2 AR.[6–8] Recently an association study[9] demonstrated linkage disequilibrium between a *Ban* I restriction fragment length polymorphism (RFLP) of the human β-2 AR gene and essential hypertension in 175 subjects. In African Americans, salt sensitivity may be linked also to the β-2 AR locus.[10] We were able to demonstrate an association of the glycine (Gly)16 variant with hypertension in 136 hypertensive and 81 normotensive African Carribeans.[11] This study provided concrete evidence implicating a functionally altered variant of the β-2 AR with hypertension. Because in the studies by Svetky et al[9] β-2 AR genotypes were defined by means of RFLP and no information concerning the site of the RFLP was revealed, a direct comparison with the results obtained in the African Caribbean subjects is not possible. In the Bergen Blood Pressure Study, an association of the β-2 AR gene and hypertension was recently shown. In this study, the arginine(Arg)16 variant was significantly more frequent in first-born offspring of 2 hypertensive parents compared with first-born offspring of 2 normotensive parents.[12] The phenotype in the Bergen Blood Pressure Study was not hypertension but rather the state of having 2 hypertensive parents

Received January 4, 1999; first decision January 27, 1999; accepted January 27, 1999.

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compared with 2 normotensive parents. Because the offspring of 2 hypertensive parents are at greater risk for the development of essential hypertension, this study provides circumstantial evidence for a possible role of the β-2 AR in the genetics of essential hypertension also in white Europeans.

A number of naturally occurring β-2 AR variants have been identified, some of which impart distinct functional properties.[13–15] Studies in transfected Chinese hamster ovary cells[16] and primary human airway smooth muscle cells[14] have shown that cells expressing the Arg16 β-2 AR form showed attenuated downregulation after β-2 agonist exposure. Conversely, cells expressing the Gly16 β-2 AR variant showed enhanced receptor downregulation.[17] This increased in vitro downregulation in response to agonists raised the hypothesis that subjects may differ in terms of cardiac and blood pressure responses according to their Arg16/Gly16 β-2 AR genotypes. We therefore conducted noninvasive hemodynamic experiments in young normotensive men who were subjected to an infusion of the selective β-2 AR agonist salbutamol.

Methods

Fifty-seven healthy Austrian Caucasian male volunteers, 20 to 32 years of age, were genotyped for the Arg16/Gly16 allele. Because hypertension is so rare in this age group, no subject had hypertension and no subjects were excluded during enrollment for this study. All homozygote subjects and 27 of 30 heterozygotes were investigated while ingesting their usual high salt intake of between 8 and 12 g salt per day. The salt intake was estimated with 24-hour urinary sodium excretion. The body mass index ranged between 18.7 and 29.9 kg/m^2. The protocol was approved by the university's committee on human subjects, and written informed consent was obtained. The subjects were characterized hemodynamically and the results analyzed with respect to the Gly16/Arg16 polymorphism of the β-2 AR. The investigators performing the hemodynamic studies were unaware of the subjects' genotypes.

Allele-specific polymerase chain reaction (PCR) procedures were developed to genotype the Arg16/Gly16 variant of the β-2 AR gene. Primers were designed using the DNASTAR software (Lasergene), referring to the gene sequence described by Kobilka et al.[17] The polymorphism was analyzed with forward primer 5′CTTCTTGCTGGCACCCAATA3′ for detection of the Arg16 allele and the same primer, substituting the A at the 3′ end for a G, for detection of the Gly16 allele. Either of these primers was combined with the reverse primer 5′ATGGAAGCGGCCCTCAGATTTGTC3′ to amplify a 697 base pair PCR product. Allele-specific PCR genotyping procedures were optimized and validated with the use of sequence data obtained in the course of a more extensive β-2 AR gene "multiplex PCR sequencing" program as a reference system. Details on multiplex PCR sequencing and the reaction conditions are given elsewhere.[12]

We used the Task Force Monitor (CNSystems, Graz, Austria), which includes real time beat-to-beat stroke volume measurements by impedance cardiography and beat-to-beat blood pressure measurements by the vascular unloading technique so that beat-to-beat changes of total peripheral resistance can be evaluated. In addition, oscillometric blood pressure recording was performed on the contralateral upper arm with the Dinamap 845. Impedance cardiography was performed by standard methods.[18] A constant sinusoidal alternating current I_0 of 400 μA and 40 kHz is passed through the thorax between a circular electrode placed around the neck and another electrode placed around the lower thorax aperture. The voltage u(t) is acquired by 2 further electrodes placed between the admitting electrodes, each at a distance of at least 3 cm from the outer electrodes to produce an interelectrode homogeneous current. The 4 electrodes consisted of aluminum tape (3M, Scotch electrical tape No. 1170), which is mounted on adhesive tape. The detected voltage u(t) is proportional to the thorax impedance Z ($Z(t)=u(t)\cdot I_0$). The first derivative (dZ/dt) of the impedance signal Z(t) is supplied analog by the impedance cardiograph. The phonocardiogram was recorded by a heart sound microphone (Hellige). The optimal placement of the microphone was evaluated with the use of a stethoscope to detect the maximal amplitude of heart sound II (usually close to the second left parasternal notch). The electrocardiogram was derived from 2 separate adhesive monitoring electrodes (3M, Red Dot 2239) that are placed on the thorax to give maximal amplitude of the R wave. The signal flow, the used algorithms for detecting heart sound II and the components of the impedance cardiogram, and for calibrating the finger blood pressure signal to the oscillometric blood pressure measurement were recently described.[19] Standard formulas were used for calculating of stroke volume and total peripheral resistance index (TPRI).[19] Stroke volume was calculated according to Kubicek et al.[18] TPRI was calculated according to Ohm's law: TPRI=MABP/CI (MABP is mean arterial blood pressure and CI is cardiac index).

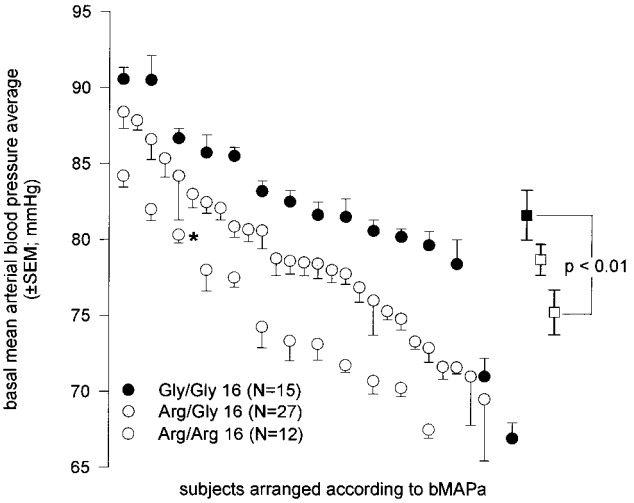

Figure 1. bMAPa with SEM in subjects homozygote for Gly16 and Arg16 allele and in heterozygotes. One subject (*) could not be included in the hemodynamic study because of frequent premature supraventricular beats. Subjects are arranged according to bMAPa to show individual SEMs.

After an overnight fast the subjects were investigated after 15 minutes of supine bed rest. Thereafter, hemodynamic monitoring was begun. First, 30 minutes were recorded for the assessment of basal hemodynamics. Then, an infusion of 0.07, 0.14, and 0.21 μg/kg per minute salbutamol, each over 8 minutes, was started. The last 4 minutes of each infusion step were used for the assessment of salbutamol-induced changes of hemodynamics. Analysis of the hemodynamic investigation proved impossible in 1 subject because of frequent premature supraventricular beats, which precluded an analysis of the impedance cardiogram. However, the subject is used for the assessment of basal blood pressure and is indicated in Figure 1.

The sample size necessary to give a level of $P<0.01$ and a power of 80% for the comparison of TPRI responses induced by salbutamol infusion was calculated from a pilot study of 7 Gly16 homozygotes and 5 Arg16 homozygotes. This calculation revealed the necessity to study 14 subjects homozygous for the Gly16 and 10 subjects homozygous for the Arg16 allele, respectively. Differences between the groups were assessed with the unpaired *t* test.

Results

A total of 57 subjects were genotyped for the Arg16/Gly16 β-2 AR alleles. The observed allele frequencies of the β-2 AR gene did not deviate from the Hardy-Weinberg equilibrium ($\chi^2=0.176$; $P=0.67$). All 15 Gly16 homozygotes, all 12 Arg16 homozygotes, and 27 of 30 heterozygotes underwent hemodynamic measurements. The Table shows the clinical

Clinical Characteristics and Basal Hemodynamics According to Gly16/Arg16 Polymorphism

	Gly16/Gly16 (n=15)	Arg16/Arg16 (n=12)	Arg16/Gly16 (n=27)
Age, y	27±3.3	27±3.2	25±3.6
Body mass index, kg/m^2	23.2±2.98	24.3±2.41	22.9±2.58
24-hour Urinary sodium, mmol/d	202.9±50.75	179.5±39.15	192.4±77.45
bMAPa, mm Hg	81.6±6.14	75.2±4.93 *	78.7±5.15
Basal systolic blood pressure average, mm Hg	115.6±6.68	110.7±7.58	112.4±6.91
Basal diastolic blood pressure average, mm Hg	62.0±7.72	55.8±6.18 †	59.1±5.57
HR, min^{-1}	61.9±6.96	58.4±6.79	60.9±8.75
SI, mL · m^{-2}	44.8±9.12	40.9±8.16‡	43.3±13.22
CI, L · min^{-1} · m^{-2}	2.7±0.49	2.4±0.48‡	2.6±0.89
TPRI, mm Hg · L^{-1} · min · m^2	30.9±6.97	32.9±7.54‡	33.0±9.90

Clinical characteristics and basal hemodynamics in subjects according to β-2 adrenoceptor genotypes.

Values are mean±SD.

*$P<0.01$; †$P<0.05$.

‡One subject had to be excluded from the hemodynamic study because of frequent premature supraventricular beats.

characteristics and basal hemodynamics according to the Gly16/Arg16 polymorphism. Subjects homozygous for the Gly16 allele show a significantly higher basal mean blood pressure average measured by an automatic oscillometric method compared with subjects homozygous for the Arg16 allele (mean±SD 81.6±6.14 versus 75.2±4.93 mm Hg; $P<0.01$; group difference 6.4 mm Hg; 95% confidence interval 1.7 to 11.1 mm Hg). Heterozygous subjects had a basal MABP average (bMAPa) in between the 2 homozygous groups. Figure 1 shows the bMAPa with the individual SEM in subjects homozygote for the Gly16 and Arg16 allele and in heterozygotes, respectively. Figure 2 shows the distribution of bMAPa, which does not deviate from a normal distribution ($\chi^2=4.6$, $P=0.71$, $df=7$). As can be seen, subjects with the Gly16/Gly16 allele are distributed in the upper range and subjects with the Arg16/Arg16 allele in the lower range of blood pressure distribution.

Figure 3 shows a representative example of the original recording of beat-to-beat heart rate (HR), stroke index (SI), CI, MABP, and TPRI from an Arg16/Arg16 subject. During each step of the salbutamol infusion, TPRI fell continuously, HR and SI increased, so that MABP remained unchanged over the infusions. Hemodynamic changes in subjects with the Gly16/Gly16, Arg16/Arg16 genotype and in heterozygotes are shown in Figure 4, A through D. SI (Figure 4A) increased incrementally in the 3 allelic groups with increasing doses of salbutamol infusion; however, the variability was such that no significant differences accrued. HR (Figure 4B) increased incrementally as well. The increases were greater in the Arg16/Arg16 group compared with the other groups. The same was true (Figure 4C) for CI. TPRI (Figure 4D) decreased with salbutamol infusion; however, the decrease was greater in the Arg16/Arg16 group compared with the other 2 groups. MABP (Figure 4E) remained unchanged in all 3 groups.

Discussion

The important findings in this study are that young adult subjects with the Gly16/Gly16 β-2 AR genotype had a significantly higher resting MABP than subjects with the Arg16/Arg16 genotype, whereas heterozygous subjects had blood pressures in between these 2 groups. Second, in

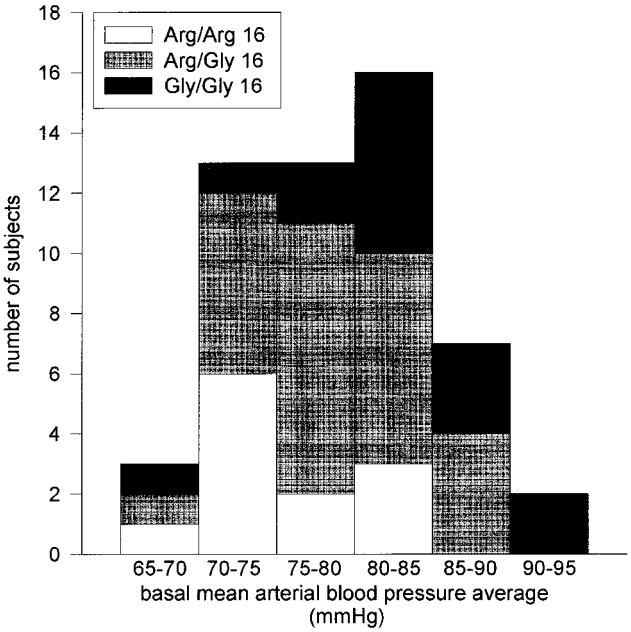

Figure 2. Basal mean arterial blood pressure average distribution in subjects according to Gly16/Arg16 polymorphism.

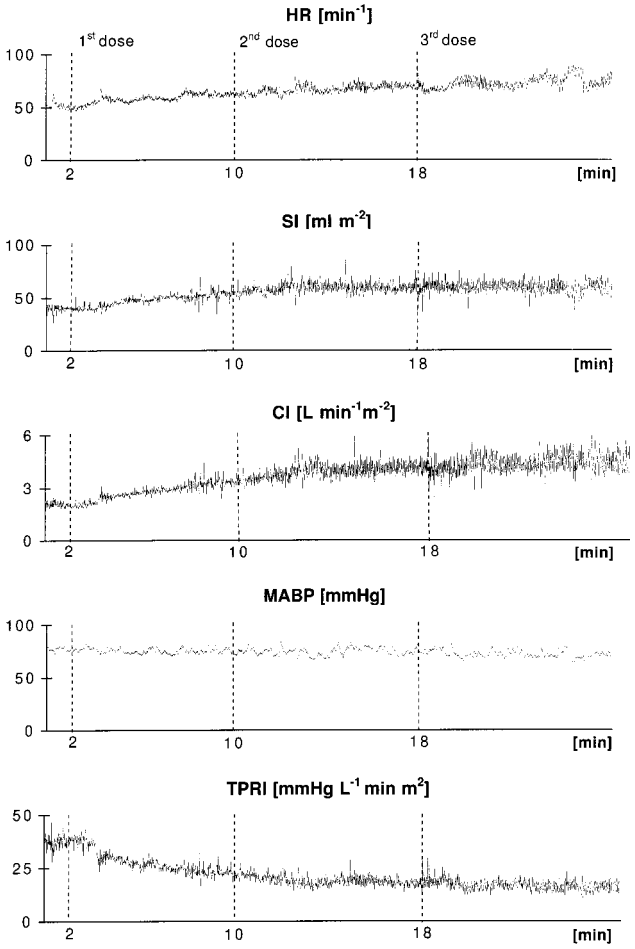

Figure 3. Original tracing of hemodynamic recording with the Task Force monitor in an Arg16/Arg16 subject during supine bed rest and during graded infusion of salbutamol.

Arg16/Arg16 individuals salbutamol infusion resulted in a stepwise greater increase in HR and CI as well as a stepwise greater decrease in TPRI compared with Gly16/Gly16 or heterozygous subjects. The effects were apparent despite baroreceptor reflex counterregulation. These data are consistent with the notion that Arg16/Gly16 genotypes respond differently to β-2 AR stimulation. The Arg16 allele appears to be associated with a greater degree of vasodilatory response compared with the Gly16 allele. This finding may be related to an increased agonist-induced downregulation of the β-2 AR in persons harboring the Gly16 variant.

Lang et al[5] observed that forearm blood-flow responses to isoproterenol were markedly attenuated in normotensive black compared with white subjects, indicating a blunting of vasodilation mediated by the β-2 AR. The authors concluded that such responses could contribute to enhanced vascular reactivity and may play a part in the pathogenesis of hypertension in blacks. These findings are especially germane because Svetky et al[10] described an association between the β-2 AR gene and salt sensitivity in African Americans and because we observed an increased frequency of the Gly16 allele in hypertensive African Caribbeans.[11] The β-2 AR gene has been considered a candidate gene for the development of essential hypertension because the β-2 AR mediates vasodilatation and because reduced vasodilatation has been found in human hypertension as well as in different animal models of hypertension.[5,10–12] Furthermore we previously reported disturbed α-2 and β-2 AR regulation[8] and a reduced β-2 AR expression on primary cultured fibroblasts in salt-sensitive compared with salt-resistant subjects.[20] The present study was conducted in healthy Austrian Caucasians, suggesting that differences in β-2 AR responsiveness are relevant irrespective of ethnic background.

Because we were interested in the overall response of the vascular bed responsible for in vivo TPRI, we used a systemic infusion of a highly selective β-2 AR agonist. We are aware of only 1 study on the effect of β-2 AR variants on vascular response to β-2 AR agonist infusion.[21] Subjects homozygous for the prodownregulatory Gln27 genotype had lower baseline forearm blood flow compared with subjects homozygous for the Glu27 variant, which is resistant to agonist-mediated downregulation in vitro.[16] The forearm blood flow response to isoproterenol was also attenuated in the Gln27 homozygotes. This study provided evidence for a relation between the Gln-Glu27 β-2 AR polymorphism and forearm vascular responsiveness to isoproterenol in a group of male normotensive subjects.

In a cohort of 324 white Europeans, linkage disequilibrium between amino acid substitutions at positions 16, 27, and 164 was observed,[22] so that 14% of those subjects with Gly16 also had the Gln27 allele compared with 52% of those subjects with Arg16. The functional properties of the haplotypes arising from variants at positions 16 and 27 have not been studied in detail; however, from the studies in transfected Chinese hamster fibroblasts[16] the (Gly16+Glu27) haplotype displayed a greater degree of agonist-mediated β-2 AR downregulation than did the (Arg16+Gln27) haplotype. Interestingly, the homozygous (Gly16+Glu27) haplotype was found to be markedly (odds ratio 10.3) overrepresented in obese subjects.[23] Future studies will be necessary to elucidate the effect of β-2 AR variant haplotypes on vascular responses to receptor agonists.

We are confident of the biological significance of our observations because we observed a difference in HR and CI between the Gly16/Gly16 and Arg16/Arg16 individuals, which indicates different sympathetic counterregulation. We speculate that the differences between Gly16/Gly16 and Arg16/Arg16 individuals would have been even greater had we blocked these counterregulatory responses. The counterregulatory response was greater in the Arg16/Arg16 individuals, so we can also assume enhanced sympathetic counterregulation at the resistance vessels. Despite the counterregulatory response, vasodilation was still greater in the Arg16/Arg16 individuals. An approach in future studies might include ganglionic blockade in the salbutamol infusion protocol.[24]

An unexpected finding was the difference in bMAPa between the Gly16/Gly16 and Arg16/Arg16 groups. It should be emphasized that in selecting the volunteers for the study, no subject had to be excluded because of hypertension. This is probably because of the rare occurrence of hypertension in this age group. Therefore the subject sample should be fairly representative for an unselected white male population. It remains to be shown whether subjects homozygotic for the

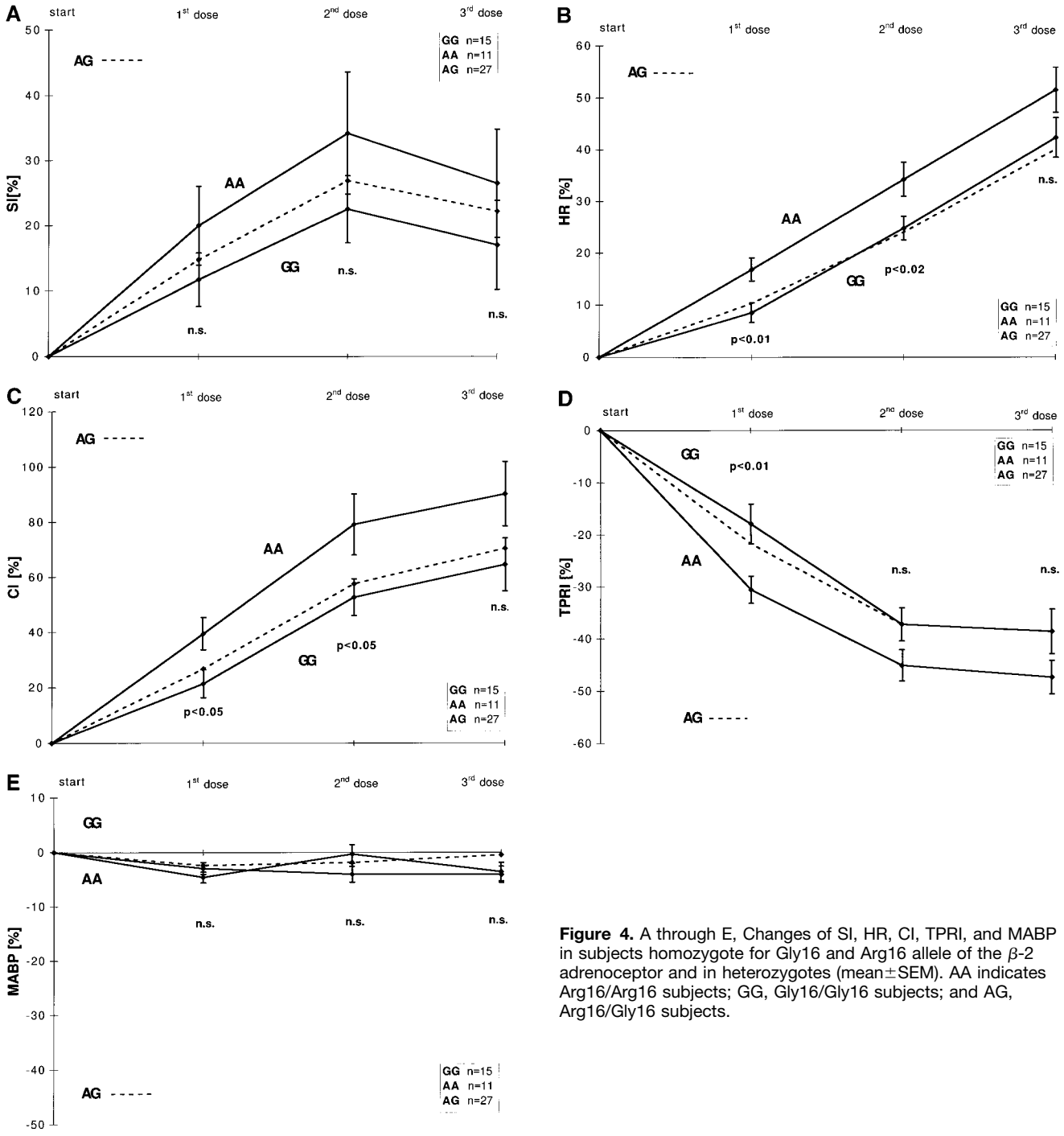

Figure 4. A through E, Changes of SI, HR, CI, TPRI, and MABP in subjects homozygote for Gly16 and Arg16 allele of the β-2 adrenoceptor and in heterozygotes (mean±SEM). AA indicates Arg16/Arg16 subjects; GG, Gly16/Gly16 subjects; and AG, Arg16/Gly16 subjects.

Gly16 genotype develop hypertension at a higher rate than those subjects homozygotic for the Arg16 genotype as could be anticipated from their higher present blood pressure. We were able to detect small differences because of the standardization applied as well as the reliance on the mean of 30 individual oscillometric blood pressure measurements. We introduced this technique in earlier studies calling for a great deal of precision.[25,26] We have shown previously that the basal blood pressure average is highly reproducible many weeks apart.[27] The higher blood pressure in the Gly16/Gly16 group is apparently not caused by a raised TPRI. Instead, the blood pressure increase appears to be caused by a marginally increased HR and CI. If the paradigm proposed by Widimsky et al[28] and Julius et al[29] is correct, these subjects may later develop hypertension not only by enhanced central nervous sympathetic stimulation but also on the basis of reduced β-2–mediated vasodilatation. However, we cannot predict possible subsequent events. In the Bergen Blood Pressure study reported recently,[12] a preponderance of hypertensive-parent offspring carried the Arg16 allele compared with normotensive-parent offspring, who had a preponderance of the Gly16 allele. We have no immediate explanation for the discrepancy but we cannot exclude a founder effect because Norwegians in Bergen may represent a relatively isolated

population. The long-term significance of our findings is also unclear.

In summary, we conclude that the Gly16 allele of the β-2 AR leads to decreased agonist-mediated in vivo vasodilatation in normotensive subjects. We suggest that the hemodynamic differences between Gly16/Gly16 and Arg16/Arg16 individuals may be of relevance for the development of essential hypertension. Further studies along these lines in normotensive and hypertensive subjects of different ethnic backgrounds will be of interest. Finally, our approach demonstrates the power of "bottom up" association studies, in which a comparatively small number of subjects are genotyped first and then phenotyped and analyzed according to their genotype.

Acknowledgments

This work was supported by the Fonds zur Förderung der Wissenschaftlichen Forschung, Austria, Sonderforschungsbreich "Biomembranes" SFB 007, and a grant-in-aid from the German Bundesministerium für Bildung und Forschung.

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