Arg16/Gly β_2-adrenergic receptor polymorphism alters the cardiac output response to isometric exercise

John H. Eisenach,1 Sunni A. Barnes,2 Tasha L. Pike,1 Lynn A. Sokolnicki,1 Shizue Masuki,1 Niki M. Dietz,1 Kent H. Rehfeldt,1 Stephen T. Turner,3 and Michael J. Joyner1

Departments of 1Anesthesiology, 2Biostatistics, and 3Internal Medicine, Mayo Clinic College of Medicine, Rochester, Minnesota

Submitted 25 April 2005; accepted in final form 28 June 2005

Eisenach, John H., Sunni A. Barnes, Tasha L. Pike, Lynn A. Sokolnicki, Shizue Masuki, Niki M. Dietz, Kent H. Rehfeldt, Stephen T. Turner, and Michael J. Joyner. Arg16/Gly β_2-adrenergic receptor polymorphism alters the cardiac output response to isometric exercise. J Appl Physiol 99: 1776–1781, 2005. First published June 30, 2005; doi:10.1152/japplphysiol.00469.2005.—Normotensive adults homozygous for glycine (Gly) of the Arg16/Gly β_2-adrenergic-receptor polymorphism have 1) greater forearm β_2-receptor mediated vasodilation and 2) a higher heart rate (HR) response to isometric handgrip than arginine (Arg) homozygotes. To test the hypothesis that the higher HR response in Gly16 subjects serves to maintain the pressor response [increased cardiac output (CO)] in the setting of augmented peripheral vasodilation to endogenous catecholamines, we measured continuous HR (ECG), arterial pressure (Finapres), and CO (transthoracic echocardiography) during isometric, 40% submaximal handgrip to fatigue in healthy human subjects homozygous for Gly (n = 30; mean age ± SE: 30 ± 1.2, 13 women) and Arg (n = 17, age 30 ± 1.6, 11 women). Resting data were similar between groups. Handgrip produced similar increases in arterial pressure and venous norepinephrine and epinephrine concentrations; however, HR increased more in the Gly group (60.1 ± 4.3% increase from baseline vs. 45.5 ± 3.9%, P = 0.03), and this caused CO to be higher (Gly: 7.6 ± 0.3 l/min vs. Arg: 6.5 ± 0.3 l/min, P = 0.03), whereas the decrease in systemic vascular resistance in the Gly group did not reach significance (P = 0.09). We conclude that Gly16 homozygotes generate a higher CO to maintain the pressor response to handgrip. The influence of polymorphic variants in the β_2-adrenergic receptor gene on the cardiovascular response to sympathoexcitatory stimuli may have important implications in the development of hypertension and heart failure.

β_2-adrenergic receptors; hypertension; genomics; sympathetic nervous system

Growing evidence suggests an association of environmental stress with the development of hypertension, and there is strong evidence in normotensive subjects that a greater pressor response to sympathoexcitatory stress is a harbinger of future hypertension (15, 17). The β_2-adrenergic receptor (β_2-AR) gene polymorphism, encoding the 16th amino acid (glycine or arginine), is associated with altered physiological and pharmacological responses to β_2-AR-mediated stimulation, suggesting that genetic influences on these intermediate physiological responses may influence the development of hypertension and perhaps heart failure (7, 24).

In healthy, normotensive Gly16 homozygotes, there is a greater forearm blood flow (FBF) response to intra-arterial infusion of a β-agonist than in Arg16 homozygotes, and the difference appears to be mediated by the endothelial generation of nitric oxide (8). This is in part consistent with findings in studies of FBF (3) and dorsal hand vein (3, 4). Additionally, in the heart, healthy normotensive Gly16 homozygotes display greater left ventricular systolic function (7, 24), which may modulate the progression to heart failure in patients with idiopathic dilated cardiomyopathy (7).

In this context, we recently reported that the higher heart rate (HR) and pressor response to mental stress and the cold pressor test were similar between Arg16 and Gly16 homozygotes. However, during isometric handgrip, the pressor response was similar, but a greater increase in HR was found in the Gly16 homozygotes, which may serve to maintain the pressor response via increased cardiac output (CO) in the face of augmented peripheral vasodilation (6). Therefore, the purpose of this study was to conduct a similar but separate study, to determine the CO and systemic vascular resistance responses to isometric handgrip to fatigue in healthy human subjects homozygous for the Gly16 or Arg16 allele. We hypothesized that Gly16 homozygotes would demonstrate a higher CO and a lower systemic vascular resistance response to isometric handgrip, and the venous catecholamine responses would be similar between groups.

METHODS

Subjects

This study was approved by the Institutional Review Board. Between July and December, 2004, 47 normotensive, unrelated volunteers (24 women, 23 men; 44 Caucasian-Americans, 1 Asian-American man, 1 Hispanic/Latino man, and 1 East Indian man) between the ages of 21 and 49 gave written, informed consent to participate. Twelve subjects in the Arg16 group and 13 subjects in the Gly16 group were participants in the previous study (6), and the remainder was recruited on the basis of genotyping performed in the interim. Candidates were considered ineligible if they were men over age 40 or women over age 50 (or postmenopausal), used tobacco products, or had any acute or chronic disorders associated with alterations in cardiovascular function. Female volunteers had a negative pregnancy test within 48 h of being studied. All women were studied in the early follicular phase of the menstrual cycle or in the low-hormone phase of oral contraceptives to minimize variability in autonomic control of cardiovascular function due to reproductive hormones (2, 18).

Genotype

On the basis of the measured Arg16/Gly genotype, subjects were placed into two groups: homozygous for the Arg16 (n = 17) or Gly16
variant (n = 30), genotyped by amplification of the relevant fragment from genomic DNA by polymerase chain reaction as previously described (8). Furthermore, the genotype of amino acid position 27 (glutamine27/glutamate, Gln27/Glu) was available for secondary analysis of the influence of this polymorphism on the results. The investigators who performed the physiology studies and processed the raw data were blinded to subject genotype.

**Protocol**

Subjects abstained from caffeine, exercise, and heavy meals on the day of study, and no alcohol was permitted for 24 h before the study. They also fasted for at least 2 h before the study. The protocol was conducted either at 10 AM (one subject), 12 PM, 2 PM, or 4 PM. The laboratory temperature was maintained between 21 and 23°C. Subjects were placed in a recumbent chair with the head and chest elevated at a 30° angle. A 16-gauge catheter was placed in a right antecubital vein for venous blood sampling. After the subject had been seated quietly for at least 10 min, three blood pressure readings taken 2 min apart were measured by automated oscillometric cuff and recorded. The readings were averaged and reported as the subjects’ baseline blood pressure.

**Measurements.** Beat-by-beat arterial pressure was measured using finger plethysmography (Finapres) and verified by oscillometric cuff before the handgrip trial. Heart rate was measured from a three-lead ECG. To ensure against breath holding in the subjects, respiratory rate was monitored with a pneumobelt. CO was measured by transthoracic echocardiography by one investigator (K. H. Rehfeldt). Venous samples were obtained at rest and during the final minute of handgrip for determination of plasma epinephrine and norepinephrine concentrations via high-performance liquid chromatography (18).

Subjects were placed in the left lateral decubitus position to optimize the echocardiography window, and the handgrip protocol was performed as described in detail previously (6). Briefly, after a 2-min baseline, each subject performed isometric handgrip with a Stoebling handgrip dynamometer (Stoebling, Wood Dale, IL) at a force of contraction that was calculated as 40% of the maximum. Because peak cardiovascular responses have been shown to occur at exhaustion, exhaustion was defined as an inability to maintain force within 10% of the target force, as described by Seals (22). Just before releasing the contraction, an ipsilateral upper arm cuff was inflated to suprasystolic level (250 mmHg) for 90 s. This postexercise circulatory occlusion (PECO) was done to trap the reflex-activating metabolites and was reported instead of systolic or diastolic pressure because of ECG. MAP was obtained from the finger plethysmography waveform and verified by oscillometric cuff and auscultatory methods.

Data were digitized at 200 Hz and analyzed offline (WinDaq; Dataq Instruments, Akron, OH). HR was obtained from the ECG. MAP was obtained from the finger plethysmography waveform and was reported instead of systolic or diastolic pressure because of more reliable tracking of MAP with Finapres during sympathoexcitation (12). Stroke volume (SV) was obtained from the left ventricular outflow tract cross-sectional area multiplied by the velocity-time integral, and CO was derived from HR and SV. Systemic vascular resistance (SVR) was calculated (MAPCO) and multiplied by 80 for conversion to dyn·s·cm⁻⁵.

**Statistics.** The sample sizes of n = 17 in the Arg16 group and n = 30 in the Gly16 group provided 80% power to detect an effect size of 0.87 (large effect) for all two-way comparisons of genotype. For subject characteristics (Table 1), the two groups were compared by the Wilcoxon rank-sum test for all variables except gender, which was compared by Fisher’s exact test. Repeated-measures ANOVA was used to assess differences between groups across various time points during handgrip. For these analyses, HR, MAP, CO, SV, and SVR were dependent variables, genotype was the independent cross-classification variable, time was the repeated factor, and a genotype-by-time interaction was included. The analysis of the venous catecholamines was done by using Wilcoxon rank-sum tests to compare the baseline measurements and the change from baseline during handgrip between the two genotype groups. Data were presented as means ± SE. Significance was set at P < 0.05.

**RESULTS**

The average duration of handgrip to exhaustion was 179 ± 10 s for Gly16 homozygotes and 169 ± 9 s for Arg16 homozygotes. Figure 1A displays the MAP responses during handgrip, at each 30 s of PECO, and 2 min of recovery. Handgrip exercise produced a significant increase in MAP (P < 0.001, main effect of time), and the increase in MAP throughout exercise was similar between groups when controlling for the baseline measurements (P = 0.96). Interestingly, during PECO, the Gly16 group did demonstrate a greater MAP (P = 0.05 genotype-by-time interaction), but this was not significant when expressed as a change from baseline. During recovery, there was no difference in MAP on the basis of genotype.

Handgrip exercise produced a significant increase in HR in all subjects (P < 0.001, main effect of time, Fig. 1B). The Gly16 group displayed a greater HR increase than the Arg16 group (P = 0.03, main effect of genotype). During PECO, the HR response was similar between groups (P = 0.74), whereas during recovery HR tended to decrease further in the Gly16 group (P = 0.07, main effect of genotype).

Handgrip exercise produced a significant increase in CO in all subjects (P < 0.001, main effect of time, Fig. 2A), and the Gly16 group displayed a greater CO response than the Arg16 group (P = 0.03, main effect of genotype). During PECO and recovery, the CO response was similar between groups (P = 0.41, 0.12, respectively).

From baseline, SVR decreased in both groups during handgrip and increased during PECO (P < 0.05, main effect of time for both). SVR was slightly lower in the Gly16 group during handgrip, but this was nonsignificant (P = 0.09, main effect of genotype, Fig. 2B). During PECO and recovery, the SVR response was similar between groups (P = 0.27, 0.79, respectively). Figure 3 displays the group average SV, with no

<table>
<thead>
<tr>
<th>Table 1. Subject characteristics</th>
<th>Homozygous Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male/female</td>
<td>Arg16 (n = 17)</td>
</tr>
<tr>
<td></td>
<td>Gly16 (n = 30)</td>
</tr>
<tr>
<td>Age, years</td>
<td>P</td>
</tr>
<tr>
<td>30 ± 1.6</td>
<td>0.23</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>113.2 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>117.1 ± 1.6</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>68.8 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>70.1 ± 1.0</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>83.6 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>85.8 ± 1.1</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>62.5 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>58.5 ± 2.1</td>
</tr>
<tr>
<td>Mean weight, kg</td>
<td>67.2 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>71.3 ± 2.2</td>
</tr>
<tr>
<td>Mean height, cm</td>
<td>168.4 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>172.1 ± 1.5</td>
</tr>
<tr>
<td>Body mass index kg/m²</td>
<td>23.6 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>24.0 ± 0.5</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>1.8 ± 0.2</td>
</tr>
</tbody>
</table>

Table entries are proportions for gender, or means ± SE for other characteristics. The 2 groups were compared by using the Wilcoxon rank-sum for all variables except gender, which was compared by using Fisher’s exact test. BP, blood pressure.
The difference in baseline SV between groups ($P = 0.14$, Wilcoxon rank-sum). The SV response was similar between the groups throughout the handgrip protocol (exercise: $P = 0.44$; PECO: $P = 0.54$; recovery: $P = 0.93$, main effects of genotype).

Venous Catecholamines

Average resting venous norepinephrine concentrations were similar between groups (Gly16: 171 ± 17 pg/ml; Arg16: 157 ± 11, $P = 0.87$). Handgrip produced a significant increase in norepinephrine that was not different on the basis of genotype (Gly16: 234 ± 17; Arg16: 243 ± 23, $P = 0.49$). Average resting venous epinephrine concentrations were similar between groups (Gly16: 16 ± 1; Arg16: 14 ± 2, $P = 0.35$). Handgrip produced a significant increase in epinephrine that was slightly greater but nonsignificant in the Gly16 group (Gly16: 49 ± 8; Arg16: 30 ± 4, $P = 0.33$). Because of this, we

---

**Fig. 1.** Mean arterial pressure (MAP; A) response to isometric handgrip at 40% maximal voluntary contraction until fatigue, followed by 90 s of postexercise circulatory occlusion (PECO) and 2 min of recovery, expressed as absolute values (top) and percent change from prestress baseline (bottom). From repeated-measures ANOVA, there was a significant genotype-by-time interaction of MAP during PECO between groups. *$P < 0.05$, Wilcoxon rank-sum test at 60 and 90 s of ischemia. However, the percent change in MAP was similar between groups. B: heart rate (HR) response was greater in the Gly16 group during the period of exercise ($P = 0.03$, main effect of genotype), and similar during PECO and recovery.

**Fig. 2.** Cardiac output (CO; A) response to isometric handgrip was greater in the Gly16 group vs. Arg16 during exercise ($P = 0.03$, main effect of genotype), but not PECO or recovery. B: systemic vascular resistance (SVR) was slightly lower but nonsignificant in the Gly16 group during the exercise ($P = 0.09$, main effect of genotype) and was similar during PECO and recovery.
and CO were greater in Gly16 than Arg16 subjects, regardless of position 27. Analysis of the HR data is consistent with findings from a previous smaller study in our laboratory (6). The physiological implications of these findings and the limitations associated with our study design will now be discussed in detail.

Interest in polymorphic variants in the \(\beta_2\)-AR is high owing to the ubiquitous presence of the \(\beta_2\)-AR across multiple effector sites that mediate intermediate physiological traits that may influence the pathogenesis and treatment of hypertension and cardiovascular disease. Experimental studies have shown that the individual coding polymorphic variants at amino acid positions 16 and 27 do not affect agonist binding affinity or downstream adenylate cyclase activity (10, 23). Early work in hamster fibroblast cells described agonist-promoted downregulation in association with the Gly16 allele and resistance to downregulation with the Glu27 allele (10). By contrast, in vivo regional vascular studies in humans have demonstrated that Gly16 homozygotes have a greater vasodilator response to \(\beta\)-agonist administration (3, 4, 8). Systemic infusion studies have shown contrasting whole body vasodilator responses in the two genotypes; however, systemic baroreflexes might mask vasodilator differences between genotypes (9, 11).

Our working hypothesis was based on the greater forearm vasodilator response to brachial artery isoproterenol in Gly16 homozygotes independent of the polymorphism at position 27 and the previous study in our laboratory that demonstrated a greater HR response to handgrip in Gly16 subjects (6, 8). The present findings confirm that both HR and CO respond to a greater extent in the Gly16 group, and the SVR response may not have reached significance because the baseline MAP was slightly higher in the Gly16 group, which persisted as a slightly higher offset during handgrip. Nonetheless, to achieve the same pressor response, greater increases in CO were needed in the Gly16 subjects, an idea consistent with the observation that Gly16 homozygotes have a greater vasodilator response to handgrip at 30% maximal voluntary contraction, which was inhibited by beta blockade (25). However, an important distinction is that the findings by Trombetta et al. (25) depended on position 27, as Gly16/Glu27 subjects had a greater FBF response in Gly16 subjects during 3 min of isometric handgrip evokes baroreceptor resetting and that a similar arterial pressure is achieved by changes in either HR or vascular resistance when the response of either is experimentally altered (14, 20). Our findings are also partially consistent with a study on Brazilian women that reported similar catecholamine levels on Brazilian men that reported similar catecholamine levels when the response of either is experimentally altered (14, 20). Our findings are also partially consistent with a study on Brazilian women that reported similar catecholamine levels and muscle sympathetic nerve activity responses in the Arg16 and Gly16 groups; furthermore, that study showed a greater FBF response in Gly16 subjects during 3 min of isometric handgrip at 30% maximal voluntary contraction, which was inhibited by beta blockade (25). However, an important distinction is that the findings by Trombetta et al. (25) depended on position 27, as Gly16/Glu27 subjects had a greater FBF response than Arg16 and Gly16/Gln27 subjects.

Taken together, the existence of other variations in the \(\beta_2\)-AR gene may influence findings in studies limited to amino acid positions 16 and 27. Analysis of 13 synonymous and nonsynonymous polymorphisms have yielded 12 \(\beta_2\)-AR haplotypes, and only 4 are common (>95% in Caucasian; >90% in other ethnicities) (5, 26). Arg16 homozygotes are nearly uniformly homozygous for Gln27, whereas subjects homozygous for Glu27 are overwhelmingly Gly16. How these haplotypes interact is important, as the aforementioned recent hand-
grip-mental stress study similar to ours showed the differences were associated with the position 27 polymorphism (25). Similar findings were also shown in a hand vein experiment by Dishy et al. (4) in which venodilator responses were greater in Gly16/Glu27 subjects than in Gly16/Gln27 and Arg16 homozygotes. However, in the present study and our previous studies, analysis of position 27 in Gly16 subjects demonstrated no evidence to suggest the Gln27/Glu polymorphism was associated with significant differences in the maximal HR and CO response to handgrip. We speculate that further understanding of the functional relevance of these polymorphic variants will require additional analysis of haplotypes, including variation in the upstream or downstream noncoding regions.

Another potential explanation of our findings is that the polymorphism may directly affect the heart during sympathoexcitatory maneuvers. We sought to determine whether the polymorphism influenced the cardiovascular response to isometric exercise from onset until fatigue. The initial signal to immediately raise arterial pressure is referred to as central command, which, through vagal withdrawal, causes a sudden increase in HR and thus CO (21). Between genotype groups, the difference in the absolute HR became more discrepant later in handgrip, similar to our previous findings (6). As subjects fatigued, epinephrine levels were significantly elevated and HR approached 100 beats/min, suggesting that cardiac sympathetic nerves were probably being activated and myocardial beta receptors stimulated, an effect that can be inhibited by beta blockade during handgrip (16).

We included the period of postexercise ischemia because we reasoned that by trapping the metabolites within the muscle upon fatigue, the pressor response would be sustained through ongoing sympathetic nerve activity (metaboreflex), whereas HR and CO would decrease (withdrawal of central command). The fact that HR was the same in each group during PECO argues that genotype interacted with a mechanism governed by central command to evoke the large increases in HR. However, during PECO, the absolute MAP was significantly greater in the Gly16 group, raising the possibility that either sympathetic activity or the effector response to sympathetic drive may also be greater in this group.

Resting SV did not reach significance between groups, an effect that may be due to the sample size in this study compared with a larger study of left ventricular indexes in these polymorphic variants (24). Nonetheless, this may have also partially contributed to the greater CO response in the Gly16 group. This idea is consistent with the previous echocardiographic findings of greater left ventricular indexes (fractionation, fractional wall shortening, midwall shortening) in normotensive but not hypertensive Gly16 vs. Arg16 homozygotes (24). Despite the predominance of β1-ARs in the myocardium, β2-ARs account for 20–30% of myocardial β-receptors, but, importantly, the ratio approaches 1:1 in heart failure, as the β1-AR undergoes downregulation relative to the β2-AR. Interestingly, in patients with heart failure, Kaye et al. (13) reported that the Arg16 polymorphism was associated with a greater HR for a given level of adrenergic drive when using the radiotracer norepinephrine spillover method. It is also possible that presynaptic β2-ARs may be present on presynaptic sympathetic nerve terminals and mediate release of norepinephrine, but this idea is controversial. In this context, we speculate that the Arg16/Gly β2-AR polymorphism may interact with nodal, presynaptic, and postsynaptic myocardial responses during exercise-mediated sympathoexcitation. Clearly, the influence of the Arg16/Gly polymorphism on ventricular performance in healthy and heart failure patients at rest and during sympathoexcitatory maneuvers deserves further study.

A limitation of studies on individual polymorphisms is the existence of other coding and noncoding polymorphic variants in the β2-AR gene that may contribute to interindividual differences in measures of intermediate physiology relevant to the regulation of blood pressure. Further characterization of additional gene sequence variation will help refine understanding of these relationships.

In summary, this study suggests that the Arg16/Gly β2-AR polymorphism can influence the cardiovascular response to isometric exercise. Consistent with a previous study in our laboratory, our findings add further support to the importance of the Arg16/Gly β2-AR polymorphism in left ventricular function and the cardiovascular responses to sympathoexcitatory maneuvers. These responses may ultimately affect how β2-AR polymorphic variants influence differences in blood pressure level and the development of hypertension. Whereas genotyping subjects for position 16 and 27 allows a good estimation of the common β2-AR haplotypes, genotyping additional sites in the β2-AR gene will allow further clarification of the phenotypic characteristics of the common haplotypes. In the future, large-scale study populations will likely undergo phenotypic screening, and the association of these traits with the common β2-AR haplotypes will be ascertained. The implications from these intermediate pleiotropic physiological observations may be substantial when applied to clinical studies of ventricular indexes and the development of hypertension and heart failure.

ACKNOWLEDGMENTS

We thank all of our subjects for their participation, and we acknowledge the excellent support of our study coordinators Pamela A. Engrav, Ruth A. Kraft, Karen P. Krucker, and Shelly K. Roberts.

GRANTS

This study was supported by National Institutes of Health Grants HL-63328, GCRC RR-00585, and NCRR K23-17520.

REFERENCES


