The Arg16/Gly β2-adrenergic receptor polymorphism is associated with altered cardiovascular responses to isometric exercise

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The β2-adrenergic receptor (β2-AR) polymorphism with glycine or arginine at amino acid position 16 (Arg16/Gly) can alter physiological and pharmacological responses to β2-AR-mediated stimulation (12, 14, 30). Interest in this polymorphism began from in vitro studies implicating it as a contributory mechanism to essential hypertension. Interestingly, some association studies have implicated the Gly allele as an important determinant in hypertension (3, 21, 29), whereas others have implicated the Arg16 allele (2, 5, 33), while still others have shown no association (16, 37). Despite these discrepancies in the causal relationship to hypertension, growing evidence suggests that the β2-AR polymorphism influences intermediate physiological characteristics relevant to the regulation of blood pressure, which may influence the susceptibility to the development of hypertension (11, 36).

In this context, the contribution of the Arg16/Gly polymorphism to differences in the pressor response to certain sympathoexcitatory maneuvers has been studied with varying results. An analysis of German Caucasian twins determined that the Arg16/Gly polymorphism was associated with systolic and diastolic blood pressure, but not heart rate (HR) at baseline, during mental arithmetic, and during the cold pressor test (CPT) (23). Conversely, a study on the pressor response during laryngoscopy and tracheal intubation in humans undergoing general anesthesia showed no association between the Arg16/Gly polymorphism and changes in arterial pressure, HR, and rate pressure product (20). However, the concurrent administration of anesthetic drugs and the highly variable responses to induction of general anesthesia and tracheal intubation for differing surgical procedures may have confounded these results.

To avoid systemic reflex responses, pharmacological studies have utilized local infusions of β2-AR agonists and demonstrated regional vasodilator responses are greater in Gly16 than Arg16 homozgyotes (6, 8). Recently, our laboratory confirmed that forearm blood flow responses to graded intra-arterial infusions of isoproterenol are greater in Gly16 than Arg16 homozgyotes and demonstrated that this difference appears to be mediated by endothelial generation of nitric oxide (11). Therefore, the purpose of this study was to measure the blood pressure and HR responses to three standard sympathoexcitatory maneuvers in healthy human subjects homozgyous for the Gly16 or Arg16 allele. We hypothesized that, compared with Arg16 homozgyotes, Gly16 homozgyotes would demonstrate a blunted pressor response to sympathoexcitatory maneuvers, which would be consistent with augmented β2-AR-mediated vasodilation in the Gly16 subjects.

METHODS

Subjects

This study was approved by the Institutional Review Board and performed in accordance with the Declaration of Helsinki. Thirty-one normotensive, unrelated volunteers (16 females, 15 males; 30 Caucasian American, 1 Asian American) between the ages of 21 and 48 gave written informed consent to participate. Candidates were considered ineligible if they were men over age 40, women over age 50 (or postmenopausal), used tobacco products, or had any acute or chronic disorders associated with alterations in cardiovascular structure or function (such as hypertension or diabetes). Candidates were also ineligible if they participated in strenuous, regular physical activity. Female volunteers had a negative pregnancy test within 48 h of being studied, and the timing of the menstrual cycle was random among all female subjects. The volunteers were taking no medica-

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tions, with the exception of oral contraceptives. Prior to the study protocol, each subject was evaluated by one of the investigators who reviewed the subject's medical history and performed a physical examination including measurement of the subject's height (by wall stadiometer), weight (by electronic balance), and blood pressure (by sphygmomanometer).

Genotype Determination

Based on the measured Arg16/Gly genotype, subjects were placed into two groups: homozygous for the Arg16 variant (n = 15) or homozygous for the Gly16 variant (n = 16). The Arg16/Gly polymorphism was genotyped by amplification of the relevant fragment from genomic DNA by polymerase chain reaction as previously described (11). Furthermore, the genotype for the polymorphism at amino acid position 27 (glutamine-27/glutamate, i.e., Gln27/Glu) was available for post hoc consideration of the influence of this polymorphism on the results. The investigators who performed the day-of-study measurements and processed the raw data (J. H. Eisenach and A. M. McGuire) were blinded to genotype of the subjects.

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 laboratory temperature was maintained between 21 and 23°C. Subjects were familiarized with the testing equipment and placed in a recumbent chair with the head and chest elevated at a 30-degree angle. Three blood pressure readings taken 2-min apart were measured by automated oscillometric cuff and recorded after the subject had been seated quietly for at least 10 min. The second and third readings were averaged and reported as the subjects’ baseline blood pressure. Following this, arterial pressure was measured on a beat-by-beat basis by finger plethysmography (Finapres) and verified by the oscillometric cuff prior to each stress trial. HR was measured from a three-lead electrocardiogram.

Stroop colored word test. Following 2 min of baseline observations, identical printed instructions were read aloud to each subject lasting 30 s. The instructions reminded subjects that best effort was required and that their performance on the test was being compared with the other subjects. A computerized version of the Stroop colored word conflict test lasting 3 min was administered to induce mental stress similar to the protocol used previously in our laboratory (7, 15, 34). To maximize the stress induced, one investigator (J. H. Eisenach) used vocally consistent monologue urging each subject to respond faster and to concentrate fully throughout the test. Data were averaged over the 3-min mental stress test and compared with averages in the 2-min prestress control period.

Cold pressor test. After 10 min of quiet rest, a 2-min baseline period was recorded, followed by a CPT as previously described by our laboratory and others (10, 28). Each subject’s right hand was immersed up to the wrist in a bucket of ice water (1–4°C), for 3 min, followed by a 2-min recovery period with the hand in a dry towel. HR and mean arterial pressure (MAP) were averaged over each minute of cold immersion and recovery; these values were compared with the average values during the prestress period immediately before immersion.

Isometric handgrip. At least 20 min before isometric exercise, each subject performed three maximal voluntary right forearm contractions (separated by 1 min each) with a Stoelting handgrip dynamometer (Stoelting, Wood Dale, IL). The force of contraction was averaged among the three maximal efforts to obtain a submaximal target, calculated as 40% of the maximum. To ensure maximal effort, subjects were encouraged by the investigators to continue squeezing until they reached fatigue. Because peak cardiovascular responses have been shown to occur at exhaustion, continuous contraction was sustained until exhaustion, defined as an inability to maintain force within 10% of the target force, as described by Seals (32). Just prior to releasing the contraction, an ipsilateral upper arm cuff was inflated to suprasystolic level (250 mmHg) for 90 s. This postexercise ischemia was done to trap the reflex-activating metabolites in the muscle following the offset of contraction, as described previously (1, 26). Upon cuff deflation, subjects rested quietly for a 2-min recovery period. To account for individual differences in time to exhaustion, the timeline was expressed as a percentage of each subject’s endurance time in 20% intervals as previously described (26). During ischemia, HR and MAP were averaged over 30-, 60-, and 90-s intervals. Data were also averaged over each minute of the 2-min recovery. Values were compared with the averages obtained from the preceding rest period.

Data Analysis

Data were digitized at 200 Hz and stored on computer. Data were analyzed offline with signal processing software (Windaq; Dataq Instruments, Akron, OH). HR was derived from the electrocardiogram waveform. MAP was derived from the finger plethysmography waveform. MAP was reported (instead of systolic or diastolic blood pressure) because previous authors have shown that, in the setting of tracking blood pressure responses during exercise or infusion of a vasoconstrictor, changes in MAP are more reliable (18, 19).

Statistics

For subject characteristics (Table 1), the two groups were compared by using the two-sample t-test for all variables except sex, which was compared by using Fisher’s exact test. Repeated measures analysis of variance (ANOVA) was used to assess differences between groups at various time points of all three sympathoexcitatory maneuvers. For these analyses, HR or MAP was the dependent variable; genotype was the independent cross-classification variable, and time was the repeated factor. Data were presented as means ± SE. Significance was set at the P < 0.05 level.

RESULTS

Mental Stress

Table 2 displays the genotype group averages for MAP and HR during the prestress period, at each of 3 min during mental stress, and during the 2-min recovery period. Mental stress produced a significant increase in MAP (P < 0.001), but there was no evidence to suggest the pattern of change was different based on genotype (P = 0.96, main effect of genotype; P = 0.42, genotype-by-time interaction). Mental stress produced a significant increase in HR (P < 0.001), but there was no

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Homozygous Group</th>
<th>Arg16 (n = 15)</th>
<th>Gly16 (n = 16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, males/females</td>
<td>9/6</td>
<td>7/9</td>
<td>0.5</td>
</tr>
<tr>
<td>Age, yr</td>
<td>30 ± 1</td>
<td>32 ± 1</td>
<td>0.4</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>117.0 ± 3.9</td>
<td>121.9 ± 2.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>70.4 ± 2.4</td>
<td>72.6 ± 2.5</td>
<td>0.5</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>85.9 ± 2.8</td>
<td>89.0 ± 2.4</td>
<td>0.4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>68.1 ± 2.0</td>
<td>64.8 ± 2.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>70.6 ± 4.1</td>
<td>73.7 ± 3.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>169.0 ± 2.3</td>
<td>173.1 ± 2.1</td>
<td>0.3</td>
</tr>
<tr>
<td>BML, kg/m²</td>
<td>24.3 ± 1.2</td>
<td>24.4 ± 0.7</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Values are counts for sex or means ± SE for other characteristics. The two groups were compared by using the two-sample t-test for all variables except sex, which was compared by using Fisher’s exact test. BP, blood pressure; MAP, mean arterial pressure; HR, heart rate; BMI, body mass index.


evidence to suggest the percent difference increased based on genotype (P = 0.44, main effect of genotype; P = 0.70, genotype-by-time interaction).

\textbf{Cold Pressor Test}

Also shown in Table 2 are average MAP and HR during the prestress period, at each of 3 min during cold immersion and during the 2-min recovery period. CPT produced a significant increase in MAP (P < 0.001), but there was no evidence to suggest this effect was different based on genotype (P = 0.45, main effect of genotype; P = 0.85, genotype-by-time interaction). CPT produced a significant increase in HR (P < 0.001), but there was no evidence to suggest this effect differed based on genotype (P = 0.55, main effect of genotype; P = 0.61, genotype-by-time interaction).

\textbf{Isometric Handgrip}

Figure 1A displays genotype group averages for MAP during the prestress period, at each 20% time interval to fatigue during handgrip, at each 30-s interval of postexercise ischemia, and during the 2-min recovery period. Isometric handgrip exercise produced a marked increase in MAP (P < 0.001), but the response between groups was very similar (P = 0.97, main effect of genotype; P = 0.69, genotype-by-time interaction). For instance, from rest to 100% fatigue, the Arg16 mean MAP increased from 88.9 ± 2.6 to 126.9 ± 3.1 mmHg, vs. the Gly16 group, which increased from 90.8 ± 2.6 to 133.2 ± 4.0 mmHg, representing a 43.5 ± 3.1% vs. 47.3 ± 3.7% increase, respectively (P \textit{interaction} = 0.43).

As shown in Fig. 1B, handgrip produced a significant HR response (P < 0.001) in all subjects, but Gly16 homozygotes displayed a greater HR response than the Arg16 group. Inclusive of all time points, there was evidence to suggest the difference in HR response was dependent on genotype (P = 0.001, genotype-by-time interaction). Furthermore, using repeated measures ANOVA on only the period of exercise (excluding forearm ischemia and recovery), the main effect of genotype on HR response was also significant (P = 0.03). Individual time points demonstrated a group difference in HR at 20% and 100% fatigue. For instance, the prestress HR for the Arg16 group was 61.5 ± 1.6, which increased to 92.7 ± 2.6 beats/min at 100% fatigue, whereas the Gly16 mean HR increased from 62.4 ± 2.5 to 99.6 ± 3.7 beats/min. From rest, this represents an increase of 42.9 ± 4.1% vs. 60.7 ± 4.3% for Arg16 and Gly16 groups, respectively (P \textit{test} = 0.005). Interestingly, the final resting HR at the second minute of recovery was lower in the Gly16 group (58.4 ± 1.5 beats/min) vs. Arg16 (63.2 ± 1.6 beats/min, P \textit{test} = 0.04). However, this was nonsignificant when expressed as a percent change from rest.

\textbf{DISCUSSION}

A major new finding of this study is that the pressor responses to all three sympathoexcitatory maneuvers did not differ significantly between Arg16 and Gly16 homozygotes. However, the glycine variant of the β2-AR polymorphism (Gly16) was associated with a greater HR response to isometric forearm exercise to fatigue compared with subjects with the Arg16 variant. These results suggest that polymorphism of the β2-AR has pleiotropic effects that are context dependent. The physiological implications of these findings and the limitations associated with our experimental design will now be discussed in detail.

There may be two possible simple explanations for the observation that HR (but not the MAP) responses were greater in the Gly16 than the Arg16 homozygotes during the handgrip trial. First, this polymorphism may directly affect HR control during sympathoexcitatory maneuvers. Despite a predominance of β1-ARs in the myocardium, β2-ARs account for 20–30% of myocardial β-receptors (4). Thus it is possible the Gly16 β2-AR polymorphism may contribute to tachycardia during sympathetic stress, an effect that may have only been seen during handgrip, since the sympathoexcitation was significantly greater than during mental stress or the CPT, consistent with a previous study comparing muscle sympathetic nerve traffic and venous norepinephrine levels in all three maneuvers (26).

The other possible explanation for the higher HRs during handgrip in the Gly16 homozygotes is that a greater β2-AR-mediated vasodilator effect was evoked by circulating catecholamines in Gly16 homozygotes. This means that to achieve the same pressor response, greater increases in HR were needed in the Gly16 subjects. This idea is consistent with the observation that 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 (22, 27). This interpretation is also consistent with findings in previous studies demonstrating greater β2-AR-mediated NO-dependent vasodilator responses during local infusion of β2-agonists in Gly16 subjects (6, 8, 11). In this context, reflex compensation might “mask” vasodilator differences between genotypes and might explain results from systemic infusion studies that have shown contrasting whole body vasodilator responses in the two genotypes (12, 17).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
 & \textbf{MAP} & \textbf{HR} & \textbf{Arg16} & \textbf{Gly16} \\
\hline
\textbf{Mental stress} & & & & \\
\textbf{Prestress} & 81.9 ± 2.6 & 85.2 ± 2.7 & 68.1 ± 2.0 & 65.2 ± 2.7 \\
1st min & 91.0 ± 3.6 & 92.4 ± 2.8 & 86.3 ± 3.5 & 82.5 ± 3.3 \\
2nd min & 98.1 ± 4.1 & 99.4 ± 3.0 & 86.4 ± 3.3 & 79.7 ± 4.2 \\
3rd min & 98.6 ± 4.1 & 99.9 ± 2.7 & 85.1 ± 3.1 & 79.2 ± 4.7 \\
1st recovery min & 86.4 ± 3.3 & 87.7 ± 2.3 & 71.0 ± 2.2 & 65.7 ± 3.1 \\
2nd recovery min & 84.8 ± 3.1 & 87.6 ± 2.7 & 68.5 ± 1.6 & 63.9 ± 2.9 \\
\hline
\textbf{Genotype} & & & 0.96 & 0.44 \\
\hline
\textbf{Interaction} & 0.42 & 0.70 & & \\
\hline
\textbf{CPT} & & & & \\
\hline
\textbf{Prestress} & 86.5 ± 2.1 & 87.5 ± 2.8 & 66.0 ± 1.7 & 62.4 ± 2.5 \\
1st min & 102.9 ± 2.4 & 104.2 ± 3.5 & 74.7 ± 2.7 & 72.9 ± 3.8 \\
2nd min & 111.8 ± 2.0 & 113.2 ± 3.3 & 71.4 ± 2.6 & 68.3 ± 3.6 \\
3rd min & 109.3 ± 2.6 & 111.5 ± 2.7 & 68.0 ± 2.3 & 67.5 ± 3.4 \\
1st recovery min & 96.9 ± 2.1 & 100.1 ± 2.8 & 61.8 ± 2.0 & 60.3 ± 2.6 \\
2nd recovery min & 90.2 ± 2.1 & 95.9 ± 3.1 & 63.6 ± 1.9 & 62.9 ± 2.3 \\
\hline
\textbf{P genotype} & & & 0.45 & 0.55 \\
\hline
\textbf{Interaction} & 0.85 & 0.61 & & \\
\hline
\end{tabular}
\caption{Cardiovascular responses to Stroop colored word test and CPT between Arg16 and Gly16 homozygote groups}
\end{table}
Fig. 1. Mean arterial pressure (MAP, A) and heart rate (HR, B) response to handgrip, 90 s of ischemia, and recovery expressed as absolute values (top), absolute change (middle), and percent change (bottom). From repeated measures ANOVA, there was no main effect of genotype on MAP in response to the maneuver, nor was there a genotype-by-time interaction. However, with HR over time, there was a significant genotype-by-time interaction ($P = 0.001$). When analyzing only the exercise segment, there was a main effect of genotype on HR ($P = 0.03$). Individual time point analyses demonstrated group differences at 20% and 100% (*$P_{\text{test}} < 0.05$). Error bars denote SE.
The existence of other variations in the β_2-AR gene may influence findings in studies examining the Arg16/Gly polymorphism. Through linkage disequilibrium, Arg16 homozygotes are nearly uniformly homozygous for Gln/Gln at amino acid position 27. For instance, Dishy et al. (8) observed that venoconstrictor responses to isoproterenol were also greater in Gly16 homozygotes; however, the differences were attributed to the effects of the Glu27 variant rather than the Arg16, because greater responses were present only in Gly16 + Glu27 homozygotes, not in Gly16 + Gln27 homozygotes, compared with Arg16 + Gln27 homozygotes (8). However, in a post hoc analysis of the Gln27/Glu polymorphism in this study, the largest difference in HR response to handgrip was observed between Arg16 + Gln27 and Gly16 + Gln27 homozygotes (peak % increase: 42.9 ± 4.1 vs. 66.9 ± 12.4%, respectively, P = 0.03). In contrast, among subjects carrying the Gly16 variant, the Gln27/Glu polymorphism was not associated with significant differences in maximal HR response to handgrip (P > 0.4). These observations support the conclusion that the Arg16/Gly polymorphism is associated with greater HR responses to isometric handgrip. Moreover, a greater HR response in Gly16 subjects would be needed to compensate augmented peripheral vasodilation if there were greater β_2-mediated vasodilator responses in the Gly16 subjects (6, 11).

Other explanations for our HR findings during handgrip are potentially complex. For example, it is possible that the Arg16/Gly β_2-AR polymorphism affects the baroreflex and/or vagal control of HR differently in these homozygous groups. Such an effect would not be manifest as a change in peripheral resistance but may entail a difference in central autonomic integration and sympathetic outflow or parasympathetic withdrawal to the heart and/or periphery.

A key question is why differing HR responses were not seen during mental stress in the two genotype groups. As noted above, the level of sympathoexcitation in these maneuvers is modest, and the subject-to-subject variability is potentially substantial (7, 31). We studied mental stress because our laboratory has shown that increased forearm blood flow seen during mental stress is mediated in part by β_2-receptor-mediated nitric oxide release (7). Since our laboratory has also shown that intra-arterial infusions of isoproterenol produce greater NO-mediated vasodilation in Gly16 homozygotes compared with Arg16 homozygotes, we hypothesized the two homozygote groups would demonstrate differences in blood pressure responses to mental stress. The absolute MAP response between groups during mental stress was similar; however, the change in MAP from prestress to mental stress was greater in the Arg16 group, albeit nonsignificant. This is supportive but not definitive evidence from the interpretation that β_2-AR-mediated vasodilation may be blunted in this group, and is consistent with findings from a larger study on normotensive, German Caucasian twins homozygous for Arg16 (n = 86) or Gly16 (n = 101) in which the diastolic response was significantly greater in the Arg16 homozygotes during mental stress (23). We speculate that our similar findings did not reach significance due to a smaller sample size, because the magnitude of difference in MAP between the two groups was almost identical in this study and the study by Li et al. (23).

Importantly, other studies have shown differing results. Subjects from the Pittsburgh Twin Study revealed no association of the Arg16/Gly polymorphism with cardiovascular responses to mental stress (25). Similarly, a previous study on the blood pressure and HR response to endotracheal intubation in Korean subjects undergoing general anesthesia was not associated with the Arg16/Gly polymorphism (20). However, it is possible that group differences in HR may have been masked by premedication with atropine, by the cholinomimetic effects of succinylcholine, the use of sedating drugs, the age range of the patients, and the ethnic differences between this subject population and ours.

Human studies have shown greater vasodilator responses to local infusions of β_2-AR agonists in Gly16 vs. Arg16 homozygotes (6, 8, 11). These findings appear, at first glance, contradictory to observations in vitro. Specifically, the Gly16 genotype is associated with enhanced agonist-induced desensitization (14), implying that β_2-AR-mediated vasodilation may be blunted in humans homozygous for Gly16. To address this apparent paradox, Liggett (24) has characterized a model of ‘‘static’’ vs. ‘‘dynamic’’ receptor regulation, postulating that the in vivo response to β_2-AR-agonists depends on whether the subject is exposed to a chronic challenge of endogenous or exogenous catecholamines. In regard to the present study, the transition from a resting state to an acute stress maneuver represents the ‘‘predesensitized’’ state consistent with the static model, where the effect of acute endogenous agonist is postulated by Liggett (24) to have no altered effect based on genotype. Our findings are at odds with this interpretation, and we suggest that the Arg16/Gly β_2-AR polymorphism influences the initial response to a sudden rise in endogenous catecholamines during handgrip, thereby producing a lower peripheral resistance, and a compensatory increase in HR in Gly16 homozygotes. Clearly, more studies are necessary to ascertain the reasons behind conflicting findings between in vivo and in vitro studies and regional vs. systemic β_2-AR-mediated vasodilation.

**Limitations**

There are limitations that deserve mention in this study. Female subjects were not studied at the same phase of their menstrual cycle. Because the diagnosis and treatment of hypertension and the response to stress is a chronic health issue, the female subjects were studied at any given point in the menstrual cycle and, when applicable, continued oral contraceptive pills prior to the study. Another limitation is the influence of coding or noncoding polymorphic variants in the β_2-AR gene may contribute to interindividual differences in measures of intermediate physiology relevant to the regulation of blood pressure. The mapping from any particular variant to these intermediate traits and, in turn, to the development of hypertension remain uncertain. Therefore, further characterization of additional DNA sequence variation in the β_2-AR gene may help refine understanding of these relationships (9, 11).

In summary, this study suggests that the Arg16/Gly β_2-AR polymorphism can influence the cardiovascular response to isometric handgrip exercise and perhaps mental stress. These findings further support growing evidence that the polymorphism may be associated with augmented β_2-AR-mediated vasodilation in Gly16 homozygotes. Such a response may mediate previously reported associations of β_2-AR polymorphisms with differences in blood pressure level and the development of hypertension. Additional studies will be needed to determine whether the β_2 genotype alters the balance between...
vasodilation and reflex control of the circulation during sympathoexcitation. Finally, although the differences reported in this and related studies may seem modest from a physiological perspective, they may have substantial implications for blood pressure regulation when applied to whole populations.

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