G Protein β3 Subunit 825T Allele and Enhanced Coronary Vasoconstriction on α2-Adrenoceptor Activation

Dietrich Baumgart,* Christoph Naber,* Michael Haude, Olaf Oldenburg, Raimund Erbel, Gerd Heusch, Winfried Siffert

Abstract—Recently, α2-adrenoceptor activation was shown to play an important role in the vasoconstriction of normal coronary arteries, whereas in the presence of atherosclerosis, the activation of both α1- and α2-adrenoceptors reduces coronary blood flow in humans. α2-Adrenoceptors activate pertussis toxin (PTX)-sensitive G proteins, whereas α1-adrenoceptors couple to PTX-insensitive G proteins. Thus, the 825T allele of the β3 subunit of heterotrimeric G proteins, associated with enhanced PTX-sensitive G protein signaling, was expected to determine the α2-adrenoceptor−, but not the α1-adrenoceptor−, mediated reduction in coronary blood flow (CBF). Genotyping was performed on 48 individuals. Twelve of the 48 received the α2-adrenoceptor agonist methoxamine (MTX; 5 mg IC), and 12 received the α2-adrenoceptor agonist BHT 933 (BHT; 5 mg IC). Twenty-four additional individuals received both MTX and BHT during the same investigational procedure. CBF was calculated on the basis of coronary angiography and intracoronary Doppler flow velocity measurement. Drug-related ischemia was assessed on the basis of ST-segment changes and angina pectoris. In response to BHT, but not to MTX, CBF was reduced to a significantly greater extent in 825T allele carriers (58±4%, n=16) than in individuals homozygous for the C825 allele (28±4%, n=19, P=0.001). This finding was independent of cholesterol levels, mean arterial blood pressure, and the presence or absence of coronary artery disease. Ischemic events in response to BHT occurred more frequently in 825T allele carriers than in homozygous 825C allele carriers (P=0.01). α2-Adrenoceptor coronary vasoconstriction is genetically determined and significantly enhanced in GNB3 825T allele carriers. (Circ Res. 1999;85:965-969.)

Key Words: proteins ■ receptor, adrenergic ■ blood flow ■ genes ■ arteries

Heterotrimeric G proteins composed of α, β, and γ subunits are important components of intracellular signal transduction and ultimately responsible for the transduction of hormonal activation of heptahelical receptors into complex physiological responses, including cell proliferation, chemotaxis, and vasoconstriction.

With the use of immortalized lymphoblasts and skin fibroblasts from patients with essential hypertension who display enhanced activation of pertussis toxin (PTX)-sensitive G proteins, we recently described a polymorphism (C825T) in the human gene GNB3 encoding for the β3 subunit of heterotrimeric G proteins (Gβ3).1 The 825T allele appears to cause alternative splicing of the gene encoding for Gβ3, thereby contributing to the generation of a functionally active splice variant, referred to as Gβ3s. Because enhanced PTX-sensitive G protein activation seemed to be the common phenotype in 825T allele carriers,1 we hypothesized that genotyping at the GNB3 825 locus allows, for the first time, the prediction of the efficacy by which hormones activate PTX-sensitive G proteins and the strength of subsequent cellular responses in humans. After this hypothesis, we recently reported that chemotaxis of neutrophils, which is mediated via βγ subunits released from PTX-sensitive G protein α subunits, is significantly enhanced in 825T allele carriers expressing Gβ3s.2 Stimulated by these findings, we became interested in whether these in vitro observations can be generalized and extended to more complex physiological in vivo responses. In this context, α2-adrenergic coronary vasoconstriction is an interesting candidate, because the involved α2-adrenoceptors represent typical heptahelical G protein–coupled receptors.3 Because the 825T allele seems to exclusively enhance PTX-sensitive G protein signaling, we hypothesized that α2-adrenergic activation, which was recently shown to occur via PTX-sensitive G proteins containing the Gβ3 subunit,4 but not α1-adrenergic activation, which occurs via PTX-insensitive G proteins, causes enhanced coronary constriction in 825T allele carriers.

We recently demonstrated in humans that in normal coronary vessels, predominantly α2-adrenoceptor activation reduces coronary blood flow (CBF), mainly through microvascular constriction.5 In the presence of atherosclerosis, both α1- and α2-adrenergic epicardial and microvascular constrict-
tions of human coronary vessels are augmented and can then induce myocardial ischemia. Although these findings account for part of the pathophysiological role of α-adrenergic coronary vasoconstriction, a common gene variation, such as the C825T polymorphism at GNB3, which determines G protein signaling, might account for the wide range of interindividual variability of α-adrenoceptor–mediated coronary constrictor responses.

Materials and Methods

Patients
Forty-eight white individuals undergoing coronary angiography due to chest pain of unknown cause were enrolled consecutively in this study after providing written informed consent. Enrollment was completed when the diagnostic coronary angiography and intravascular ultrasound examination yielded either a normal left coronary artery or single-vessel disease that was suitable for quantitative evaluation. Normal coronary arteries were defined when patients had no plaques or had plaques with <10% area stenosis in the major left coronary arteries on intravascular ultrasound examination. Demographic data are given in Table 1.

Twelve individuals received the selective α1-adrenoceptor agonist methoxamine (MTX) and 12 individuals received the selective α1-adrenoceptor agonist BHT-933 (BHT), both intracoronarily (IC) and in random order. Twenty-four additional individuals received both MTX and BHT in random order during the same investigational procedure. Individuals were divided into 4 groups depending on the α-agonist they received and on the presence or absence of coronary artery disease (CAD).

Study Protocol
The study protocol was approved by the local ethics committee of the University of Essen Medical School. With the exception of 100 mg/d aspirin, all medication was stopped 24 hours before cardiac catheterization. Aortic pressure measurement, biplane left ventricular angiography, intravascular ultrasound examination, and coronary catheterization were performed and intracoronary Doppler flow velocity measurements were made as previously described. One individual of the BHT group had to be excluded from further analysis because the quality of the Doppler signal was not satisfactory.

After the intravenous injection of 1 mg atropine to prevent reflex bradycardia, the respective α1-adrenoceptor agonists were administered in a dose of 5 mg IC each as a bolus via the guiding catheter. Based on our previous study, a dose of 5 mg of each agonist was expected to result in clear coronary vasomotor effects. Doppler flow velocity, arterial blood pressure, and ECG were recorded continuously.

Occurrence of Myocardial Ischemia
Drug-related ischemia was assumed if chest pain was reported or if ST-segment depression of ≥0.2 mV occurred on the ECG within 10 minutes after the injection of the respective drug.

Genotyping
DNA extraction and genotyping were performed in a blinded fashion as previously described.

Statistical Analysis
The results are expressed as mean±SEM. Data were analyzed with the use of the SPSS software package 8.0.0G (SPSS Inc). Categorical data were compared with the use of the χ² test or the Fisher exact test, where appropriate. An exact test was used to determine whether the null hypothesis of an odds ratio of 1 for the occurrence of ischemic events could be rejected. Intragroup variations, as well as comparisons between groups with regard to mean aortic blood pressure (MAP), heart rate (HR), and cholesterol levels, were performed with the use of the Student t test. Because several factors were reported to influence coronary vasomotion, a general linear model was used that included cholesterol levels, MAP, heart rate, and cholesterol levels, were performed with the use of the Student t test. Because several factors were reported to influence coronary vasomotion, a general linear model was used that included cholesterol levels, MAP, and cholesterol levels, were performed with the use of the Student t test.

An expanded Materials and Methods section is available online at http://www.circresaha.org.

Results
Demographic and clinical data for individuals with different genotypes at GNB3 in each drug group were comparable (Table 1). Genotyping yielded 2 individuals who were ho-

### Table 1. Baseline Data for Study Population by α1-Adrenoceptor Agonist and Genotype at GNB3 (C825T)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age, y</th>
<th>Male/Female, n</th>
<th>CAD</th>
<th>Hypertension</th>
<th>Type 2 Diabetes</th>
<th>Current Smokers</th>
<th>Serum Cholesterol, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT 5 mg IC</td>
<td>36</td>
<td>53.2±2.6</td>
<td>20/15</td>
<td>18 (51.4)</td>
<td>13 (37.1)</td>
<td>4 (11.4)</td>
<td>17 (48.6)</td>
<td>217.1±7.8</td>
</tr>
<tr>
<td>GNB3 825 CC</td>
<td>19</td>
<td>52.3±3.2</td>
<td>9/10</td>
<td>8 (42.1)</td>
<td>6 (31.6)</td>
<td>2 (10.5)</td>
<td>9 (47.4)</td>
<td>226.5±11.7</td>
</tr>
<tr>
<td>GNB3 825 TC+TT</td>
<td>16</td>
<td>53.9±3.3</td>
<td>11/5</td>
<td>10 (62.5)</td>
<td>7 (43.8)</td>
<td>1 (6.3)</td>
<td>8 (50.0)</td>
<td>207.0±10.0</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTX 5 mg IC</td>
<td>36</td>
<td>54.0±2.3</td>
<td>23/13</td>
<td>21 (58.3)</td>
<td>11 (30.6)</td>
<td>9 (25.0)</td>
<td>12 (33.3)</td>
<td>226.2±9.9</td>
</tr>
<tr>
<td>GNB3 825 CC</td>
<td>17</td>
<td>53.3±3.4</td>
<td>9/8</td>
<td>9 (52.9)</td>
<td>5 (29.4)</td>
<td>4 (23.5)</td>
<td>4 (23.5)</td>
<td>236.9±17.4</td>
</tr>
<tr>
<td>GNB3 825 TC+TT</td>
<td>19</td>
<td>55.2±2.8</td>
<td>14/5</td>
<td>12 (63.2)</td>
<td>6 (31.6)</td>
<td>3 (15.8)</td>
<td>6 (31.6)</td>
<td>217.6±11.1</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

CC/TC/TT indicates homozygosity and heterozygosity for C825 or 825T alleles at GNB3. 

P values are given for the comparison between genotypes in each medication group and were calculated as indicated in Materials and Methods. 

Values are n (%) or mean±SEM.
mozygous, 23 individuals who were heterozygous for the 825T allele, and 23 individuals who were homozygous for the 825C allele. HR and MAP values were comparable among groups at baseline and remained unchanged when the local coronary vasoconstrictor responses were measured. MAP subsequently increased, however, due to the recirculation of study drugs, and HR decreased. Under baseline conditions, average peak velocity, cross-sectional area in the Doppler segment, and CBF did not differ among groups, regardless of genotype and CAD status (Table 2).

**α₂-Adrenoceptor Activation**

The CBF reduction induced by BHT was significantly larger in 825T allele carriers than in homozygous C825 allele carriers (58 ± 4% versus 28 ± 4%, P = 0.001; Figure 1) and significantly enhanced in patients with CAD compared with patients without CAD (53 ± 5 versus 30 ± 5%, P = 0.04; Figure 2).

In individuals with the homozygous CC genotype, CBF was markedly reduced in response to α₂-adrenoceptor activation and further reduced in the presence of CAD (39 ± 8% versus 20 ± 3%, P = 0.02). Although CBF reduction was even greater in 825T allele carriers, the additional effect of CAD was rather moderate (64 ± 5% versus 48 ± 7%, P = 0.07).

In 8 patients with an average CBF reduction of 55 ± 10%, clinical signs of acute ischemia, as represented by ST-segment depression or symptoms of angina pectoris, were observed in response to BHT (Table 2). This effect was almost exclusively observed in heterozygous 825T allele carriers with (3 TC and 1 CC) and without (4 TC) CAD. The resulting odds ratio for ischemia in response to BHT was significantly increased in 825T allele carriers (odds ratio, 14.0; 95% confidence interval, 1.5 to 132; P = 0.01; Table 3).

**Figure 1.** Impact of C825T polymorphism at GNB3 on CBF reduction: response to intracoronary application of 5 mg MTX or BHT in percentage of basal values. TT/TC indicates homozygosity and heterozygosity for 825T allele carriers and CC, homozygosity for 825C allele at GNB3. Horizontal line represents mean value of respective group. P values are given from general linear model.

**Figure 2.** Impact of CAD on CBF reduction: response to intracoronary application of 5 mg MTX or BHT in percentage of basal values. Horizontal line represents mean value of respective group. P values are given from general linear model.

**TABLE 3. Clinical Signs of Ischemia in Patients Receiving BHT or MTX**

<table>
<thead>
<tr>
<th>Drug</th>
<th>TT +TC/CC</th>
<th>All</th>
<th>No Ischemia</th>
<th>Ischemia</th>
<th>OR (95% CI)</th>
<th>TT +TC vs CC</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT</td>
<td>16/19</td>
<td>9/18</td>
<td>7/1</td>
<td>1.5</td>
<td>14 (1.5–132.0)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>MTX</td>
<td>19/17</td>
<td>17/16</td>
<td>2/1</td>
<td>1.88</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CC/TC/TT indicates homozygosity and heterozygosity for C825 or 825T allele at GNB3; OR, odds ratio for the occurrence of ischemic events; and CI, confidence interval. Ischemia is defined as chest pain or ST-segment depression of ≥0.2 mV on the ECG within 10 minutes after the injection of study drug.

**Discussion**

In the experimental setting, α₂-adrenergic coronary constriction is overcome by metabolic dilation under physiological circumstances but is sufficiently powerful to reduce CBF when local regulatory mechanisms are impaired by endothelial dysfunction, atherosclerosis, autoregulatory escape, or distal to severe stenoses. In patients with CAD, isometric exercise and reflex sympathetic activation can induce ischemic myocardial dysfunction and angina pectoris, which are in part caused by α₂-adrenergic coronary vasoconstriction.

Both α₁- and α₂-adrenoceptor activation play an important role in coronary vasoconstriction. In our previous study, in normal coronary arteries, CBF was mainly reduced through the activation of α₁-adrenoceptors, whereas in atherosclerotic coronary arteries, both α₁- and α₂-adrenoceptor activation contributed equally to the reduction in blood flow.

Somewhat different from our previous investigation but consistent with the study of Lorenzoni et al., in the present study, we found a small but significant decrease in blood flow in normal human coronary vessels on α₁-adrenoceptor activation. This difference can most likely be explained from a statistical point of view, because the number of patients in the previous study was probably not sufficient to achieve significance in differentiation between several doses of the α₁-agonist. We observed a large interindividual variability in...
vasoconstrictor responses to α-adrenoceptor activation. Given the enhanced responsiveness of PTX-sensitive G proteins in individuals carrying the GNB3 825T allele, we hypothesized that a large fraction of the variability seen on α2-adrenoceptor, but not α1-adrenoceptor, activation is determined by genotype. In fact, we could now demonstrate that the presence of an 825T allele was a strong predictor of selective α2-adrenergic coronary vasoconstriction, independent of MAP, HR, and cholesterol levels. Even more remarkable was that this effect was independent of coronary artery status, which also contributed to CBF reduction in response to BHT, potentially through impaired coronary vasodilator capacity in the presence of endothelial damage. However, with the use of a descriptive analysis, the impact of CAD was markedly lower in 825T allele carriers.

As expected, no differences occurred with respect to genotype at GNB3 on selective α2-adrenoceptor activation, whereas the CBF reduction was significantly enhanced in patients with CAD, in line with our previous observations.5

In response to BHT, the 825T allele carriers also significantly more often displayed clinical signs of ischemia, which was most likely due to the greater CBF reduction, because further analysis of data from our previous study5 indicated that the decrease in myocardial lactate extraction, as an indicator of myocardial ischemia, is significantly correlated to the reduction of CBF in response to 5 mg IC of the respective study drug (Spearman’s R = 0.725, P = 0.008). Interestingly, we recently reported that the presence of an 825T allele also increases the likelihood for myocardial infarction in individuals with CAD.21 Although these findings improve our pathophysiological understanding, especially of patients with abnormal vascular reactions and angina pectoris symptoms in the absence of any flow-limiting epicardial coronary stenosis, the physiological importance of this observation remains to be defined. No investigation has been performed in vivo, with the use of norepinephrine as the endogenous adrenoceptor activator or physical activity, to evaluate the distinct impact of α1- and α2-adrenoceptors on CBF under physiological circumstances in humans.

The molecular basis of the observed association between α2-adrenergic activation and genotype at GNB3 has yet to be determined. α2-Adrenoceptors regulate vascular tone via 2 distinct mechanisms. First, they may mediate vasoconstriction through the inhibition of adenyl cyclases and activation of phospholipase C, with the latter process involving G protein βγ subunits.22,23 Second, α1-adrenoceptor activation mediates vasodilation, which is assumed to be secondary to the release of nitric oxide from the endothelium.24 Thus, one could speculate that the enhanced blood flow reduction in 825T allele carriers may be counteracted by a concomitantly increased vasodilation. However, α1-adrenoceptor activation in vivo usually causes vasoconstriction that is not overcome by vasodilation, with the latter mainly observed under in vitro conditions (eg, in preconstricted, isolated arteries). Furthermore, although α2-adrenoceptor–mediated vasodilation seems to involve nitric oxide formation via PTX-sensitive G proteins in large conductance arteries,24 in other vascular beds, such as in coronary arteries,25 cerebral arteries,26 or mesenterial resistance arteries,27 nitric oxide either seems to have no effect or inhibits α1-adrenoceptor–mediated vasodilation. Not much is known about the mechanisms of the nitric oxide–independent, α2-adrenoceptor–mediated vasodilation, although a very recent report suggests the involvement of smooth muscle Ca2+-operated K+ channels.27

Conclusions

Beyond the augmentation of α1- and α2-adrenergic coronary vasoconstriction in the presence of coronary atherosclerosis, the response to α2-adrenoceptor activation is likely genetically determined and is strongly associated with the 825T allele at GNB3. The enhanced coronary vasoconstrictor response in 825T allele carriers with and without CAD was accompanied by a higher rate of signs and symptoms of myocardial ischemia.

Acknowledgments

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References


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