G894T Polymorphism in the Endothelial Nitric Oxide Synthase Gene Is Associated With an Enhanced Vascular Responsiveness to Phenylephrine

Ivan Philip, MD; Gaetan Plantefève, MD; Sandrine Vuillaume-Barrot, PhD; Eric Vicaut, MD, PhD; Claude LeMarie, MD; Daniel Henrion, PhD; Odette Poirier, PhD; Bernard I. Levy, MD, PhD; Jean Marie Desmonts, MD; Geneviève Durand, PhD; Joelle Benessiano, PhD

Background—Differences in vascular reactivity to phenylephrine (PE) responsiveness have been largely evidenced in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB). Because nitric oxide (NO) strongly affects modulation of the vascular tone in response to vasopressor agents, we hypothesized that the G894T polymorphism of the endothelial NO synthase gene (eNOS) could be related to changes in the pressor response to PE.

Methods and Results—The protocol was performed in 68 patients undergoing coronary artery bypass grafting (n=33) or valve surgery (n=35) in whom mean arterial pressure decreased below 65 mm Hg during normothermic CPB. Under constant and nonpulsatile pump flow conditions (2 to 2.4 L • min⁻¹ • m⁻²), a PE dose-response curve was generated by the cumulative injection of individual doses of PE (25 to 500 μg). The G894T polymorphism of the eNOS gene was determined, and 3 groups were defined according to genotype (TT, GT, and GG). Groups were similar with regard to perioperative characteristics. The PE dose-dependent response was significantly higher in the allele 894T carriers (TT and GT) than in the homozygote GG group (P=0.02), independently of possible confounding variables.

Conclusions—These results evidenced an enhanced responsiveness to α-adrenergic stimulation in patients with the 894T allele in the eNOS gene. (Circulation. 1999;99:3096-3098.)

Key Words: nitric oxide • endothelium • genes • receptors, adrenergic, alpha • vasoconstriction

Hypotensive events occurring in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) frequently require vasoconstrictor therapy. Phenylephrine (PE) is an α₁-adrenoceptor agonist commonly used in such clinical settings to support stable hemodynamics. However, experimental evidence shows differences in vascular reactivity to PE during CPB that remain largely unexplained.

The endothelium plays an important part in functional changes in the vasculature by releasing physiologically active substances that locally modulate arterial tone. Both under basal conditions and after agonist or mechanical stimulation, the endothelium releases relaxing factors such as nitric oxide (NO) or NO-containing molecules.

In humans, a variant of the endothelial NO synthase (eNOS) gene within exon 7 has been identified: G→T transversion at nucleotide position 894 of eNOS cDNA, resulting in a change of Glu298 (GAG) to Asp (GAT). A significant association between the risk of coronary heart disease, especially in vasospastic angina pectoris, and the eNOS polymorphism has been found, which suggests that the G894→T variant in the eNOS gene could affect vascular coronary reactivity.

Because activation of eNOS induces the release of the potent vasodilator NO, we hypothesized that the polymorphism of the eNOS gene could be related to changes in responsiveness and vascular reactivity to vasoactive agents. The aim of this work was to determine a possible association between the G894→T polymorphism in the eNOS gene and vascular responsiveness to α-adrenergic stimulation during cardiac surgery in patients requiring vasoconstrictor therapy.

Methods

Patient Selection

The following protocol was approved by the local ethics committee, and all patients gave their informed consent. We studied 68 consecutive patients requiring PE therapy during CPB among a total of 564 patients undergoing elective coronary artery and/or valve surgery. All experiments were performed in a blinded manner. Preoperative characteristics of the patients are shown in Table 1.

Anesthesia and CPB Management

Anesthesia was induced with high doses of fentanyl, midazolam, and pancuronium bromide and maintained by repeated injections of the same agents (Table 2). Normothermic nonpulsatile CPB and myocardial protection were standardized for all patients.
TABLE 1. Preoperative Characteristics of Study Patients

<table>
<thead>
<tr>
<th>Genotype</th>
<th>TT (n=7)</th>
<th>GT (n=29)</th>
<th>GG (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>68±9</td>
<td>67±13</td>
<td>62±15</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>5/2</td>
<td>19/10</td>
<td>20/12</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>1.81±0.19</td>
<td>1.83±0.18</td>
<td>1.85±0.22</td>
</tr>
<tr>
<td>Surgery, CABG/VS</td>
<td>5/2</td>
<td>16/13</td>
<td>12/20</td>
</tr>
</tbody>
</table>

Pharmacological therapy
- β-Blockers: 4 8 12
- ACE inhibitors: 1 9 13
- Ca channel blockers: 4 7 6
- Nitrates: 4 11 9
- Normal LV function: 4 19 19
- History of hypertension: 4 10 12
- Myocardial infarction: 3 10 6
- Diabetes: 1 5 2

VS indicates valve surgery; LV, left ventricular.
Values are mean±SD. For both groups, all comparisons according to genotype were not significant.

PE Dose-Response Curve
Mean arterial pressure (MAP) was recorded through a radial artery catheter. The transducer was maintained at the midaxillary level. Intraoperative parameters are shown in Table 2. Patients with extreme values of hematocrit (<20% and >35%), pH (<7.4 and >7.6), PCO₂ (<3.0 kPa and >5.3 kPa), and temperature (<35°C) were excluded.

Within 30 minutes after the onset of CPB, after stabilization of temperature and biological parameters, baseline hemodynamic parameters were continuously recorded, and the protocol was performed when MAP decreased below 65 mm Hg for a pump flow ranging from 2 to 2.4 L·min⁻¹·m⁻². The PE dose-response curve was then generated by injection of individual doses of PE, with a 2-minute interval between injections, until MAP reached a value of 85 mm Hg (in the following sequence of 25, 50, and 75 μg). Comparisons of dose-response curves in GG versus GT and TT patients were made by 2-way ANOVA between factors (ie, genotypes) and within factor (ie, dose). An additional analysis was made to ensure that the effect of genotype was not due to confounding factors. For this purpose, a multivariate regression analysis of the area under the dose-response curve was performed, taking into account genotype and main clinical variables (ie, age, sex, previous myocardial infarction, hypertension, diabetes, treatments, and type of surgery). Because of skewed statistical distribution of the response, all analyses were performed after normalization of the logarithmic transformation of this variable.

In all univariate tests, the significance level was fixed at 5%. For the multivariate regression analysis, the threshold F value to accept a variable in the model was fixed at 4.0, as suggested by Dixon. All tests were performed with the Biomedical data package (BMDP, from UCLA at Los Angeles, Calif).

TABLE 2. Intraoperative Characteristics of Study Patients

<table>
<thead>
<tr>
<th>Anesthetic agents</th>
<th>TT (n=7)</th>
<th>GT (n=29)</th>
<th>GG (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fentanyl, μg/kg</td>
<td>97±16</td>
<td>92±26</td>
<td>87±24</td>
</tr>
<tr>
<td>Midazolam, mg/kg</td>
<td>0.12±0.07</td>
<td>0.11±0.06</td>
<td>0.11±0.05</td>
</tr>
<tr>
<td>Pump flow, L·min⁻¹·m⁻²</td>
<td>2.24±0.19</td>
<td>2.27±0.15</td>
<td>2.20±0.15</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>26.0±4.4</td>
<td>27.1±4.6</td>
<td>26.7±3.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.52±0.05</td>
<td>7.50±0.05</td>
<td>7.52±0.06</td>
</tr>
<tr>
<td>Pao₂, kPa</td>
<td>45.2±12</td>
<td>47.8±6</td>
<td>47.3±7</td>
</tr>
<tr>
<td>Paco₂, kPa</td>
<td>3.94±0.5</td>
<td>4.21±0.6</td>
<td>4.02±0.4</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>35.9±0.6</td>
<td>36.0±0.5</td>
<td>35.9±0.5</td>
</tr>
</tbody>
</table>

Values are mean±SD. For all groups, comparisons according to genotype were not significant.

Detection of the G to T Variation in the eNOS Gene
Genotyping of all subjects was performed by polymerase chain reaction amplification according to previously described procedures. Data Analysis
Data were expressed as mean±SD and analyzed within genotype groups by 2-way ANOVA or χ² test. Analyses of PE dose-response curves were performed only for the 3 doses of PE that were administered in all patients (ie, 25, 50, and 75 μg). Comparisons of dose-response curves in GG versus GT and TT patients were made by 2-way ANOVA between factors (ie, genotypes) and within factor (ie, dose). An additional analysis was made to ensure that the effect of genotype was not due to confounding factors. For this purpose, a multivariate regression analysis of the area under the dose-response curve was performed, taking into account genotype and main clinical variables (ie, age, sex, previous myocardial infarction, hypertension, diabetes, treatments, and type of surgery). Because of skewed statistical distribution of the response, all analyses were performed after normalization of the logarithmic transformation of this variable.

In all univariate tests, the significance level was fixed at 5%. For the multivariate regression analysis, the threshold F value to accept a variable in the model was fixed at 4.0, as suggested by Dixon. All tests were performed with the Biomedical data package (BMDP, from UCLA at Los Angeles, Calif).

Results
No differences between the 3 groups were found with respect to preoperative variables, pharmacological therapy, or intraoperative characteristics (Tables 1 and 2).

eNOS Genotypes
Among the 564 genotyped patients and in the 68 patients requiring PE infusion during CPB, we reported similar allelic frequencies of the 894T allele (0.48) and genotype distribution in the TT, GT, and GG groups (11%, 40%, and 49% versus 10.8%, 40%, and 49.2%, respectively), in agreement with the frequencies predicted by Hardy-Weinberg equilibrium (χ²=0.0017; P=NS).

Hemodynamic Study and PE Dose-Response Curves
Initial MAP before PE administration was similar in the 3 groups (54.1±6, 53.6±10, and 56.0±6 mm Hg in the TT, GT, and GG groups, respectively). The PE dose-dependent response was significantly higher in the allele 894T carriers (TT and GT) than in the homozygote GG group (P=0.02). Moreover, in the TT group, no patient needed a PE injection ≥150 μg to reach a MAP value of 85 mm Hg (Figure). In multivariate analysis, eNOS genotype appeared as the only predictive variable of the PE-response.

Discussion
The main finding of the present study was an enhancement of the hemodynamic response to PE in patients with the 894T allele in the eNOS gene, suggesting that the vascular responsiveness to vasoconstricting hormones could be modulated by the polymorphism of the eNOS gene in humans. The eNOS genotype distribution found in the present work was only representative of a population of adult cardiac surgery patients and not comparable to those previously reported.
In our conditions, MAP recorded in patients undergoing CPB was chosen as a marker for the vascular response to PE. Extracorporeal circulation provided a model of purely resistive vascular behavior, because pump flow can be controlled. Moreover, during nonpulsatile bypass, flow and pressure are primarily nonoscillatory, and the pressure-flow relationship is determined mainly by the vascular resistance.6

Initial MAP was similar in the 3 eNOS genotype groups, ruling out an effect of the polymorphism of the eNOS gene in measured basal blood pressure. Conversely, the enhanced hemodynamic response to bolus PE found in patients with the 894T allele, independently of perioperative factors known to affect \( \alpha \)-adrenergic responsiveness in patients undergoing CPB,7 suggested an association between the polymorphism of the eNOS gene and the responsiveness to \( \alpha \)-adrenergic stimulation.

Although our study does not identify any involved mechanism, we can hypothesize that the polymorphism of the eNOS gene could affect stimulated NO production. Thus, because in vivo and in vitro studies have demonstrated that the endothelium, by releasing NO, reduces the maximal response to PE,8 we can suggest a lesser production of NO in patients with the 894T allele. Although the G894T variant is not located in any functional consensus sequence, it could be involved in a conformation change in the eNOS protein, consequently inducing an alteration in the NO pathway.

Changes in endothelial function may have important clinical implications for the pathogenesis of cardiovascular disorders. Therefore, functional alterations of the endothelium-derived NO pathway, especially those involved in the pathogenesis of coronary spasm1 and hypertension,4 may be due to a lesser endothelial release of NO related to the presence of the 894T allelic form of the eNOS gene, as suggested by the present study.

In summary, this study reports the first evidence for an association between the G894→T polymorphism in the eNOS gene and enhanced vascular reactivity to PE in humans, suggesting that DNA sequence differences in the eNOS gene may affect vascular responsiveness and reactivity to \( \alpha \)-adrenergic stimulation. However, because the sample of the present study has been highly selected, the sample size is less than in normal gene polymorphism association studies. Thus, another independent study seems to be necessary to verify our present finding. Furthermore, it remains to be determined whether the G894T variant gives rise to direct functional alterations of the endothelium-derived NO pathway or is a genetic marker associated with some causal loci. Functional analysis of the 894T variant and measurement of the NO production must be performed to confirm the results of our study.

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References

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