Preproendothelin-1 Gene Polymorphism Is Related to a Change in Vascular Reactivity in the Human Mammary Artery In Vitro

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Abstract—A gene polymorphism of preproendothelin-1 (a G-to-T transversion that predicts a Lys/Asn change at codon 198) associated with an increased risk of hypertension has been recently described in patients carrying the T allele. No study has yet determined the impact of this polymorphism on vascular reactivity, although a functional role for endothelin-1 in the pathophysiology of hypertension has been clarified. At subthreshold concentrations, endothelin-1 and angiotensin II induce a potentiation of α-adrenergic–dependent vascular tone caused by an increased sensitivity of the contractile apparatus to calcium. We investigated phenylephrine-induced tone and its amplification by endothelin-1 and angiotensin II in human mammary artery rings in vitro. Contractions to phenylephrine (0.1 to 100 μmol) and endothelin-1 (0.1 to 300 nmol) were not significantly different in rings from GT/TT (n=27) and GG (n=21) patients. A subthreshold concentration of endothelin-1 (10 pmol) potentiated a phenylephrine-induced contraction (eg, 44±12% increase in tone with phenylephrine 1 μmol/L, P<0.001) that was significantly higher in the GT/TT group than in the GG group (eg, 44±12% versus 82±11%, P<0.01). A similar effect on response to phenylephrine was observed with a subthreshold concentration of angiotensin II. We also found a higher response to calcium in arteries from GT/TT patients. Endothelium-dependent or -independent relaxations were unaffected by the genotype. These data suggest that the preproendothelin-1 gene polymorphism is associated with a higher potentiating effect of endothelin-1 and angiotensin II, probably in relation with higher calcium sensitivity. These changes in vascular reactivity might help to understand the relations between this polymorphism and cardiovascular disorders. (Hypertension. 2002;39:209-213.)

Key Words: blood vessels ■ polymorphism ■ endothelin ■ phenylephrine ■ angiotensin II

A gene polymorphism of preproendothelin-1 has been described as a G-to-T transversion at position 5665 affecting the 61st nucleotide of exon 5, which predicts a Lys/Asn change at codon 198. Among the several epidemiological studies performed, one has shown that this polymorphism was associated with a higher blood pressure in overweight patients with the T allele.1 If gene polymorphisms have to be considered in the physiopathology of cardiovascular diseases, the knowledge of their vascular effects remains a key issue. Indeed, no study has yet been conducted to determine the functional impact of this polymorphism on vascular function. A key role for endothelin-1 in the control of vascular tone has been described in different types of blood vessels, and its role in several pathological situations has been clarified.2–13 At subthreshold concentrations, within the physiological (picomolar) range, endothelin-1 has no direct contractile effect but induces a potent amplification of α-adrenergic–dependent vascular tone.14,15 This amplification by endothelin-1 involves a sensitization of the contractile apparatus to calcium15 and has been described in animals15 and humans.14 This phenomenon represents a mechanism by which endothelin-1 can modulate vascular tone without inducing the long-lasting and irreversible contraction as classically described for endothelin-1. This potentiation phenomenon is common to several hormonal systems, such as angiotensin II.16–23 We investigated phenylephrine-induced tone and its amplification by endothelin-1 or angiotensin II (10 pmol/L). We tested the hypothesis that the preproendothelin-1 gene polymorphism might be related to changes in vascular reactivity to physiological subthreshold concentrations of endothelin-1. We used human mammary artery segments from patients undergoing cardiac bypass surgery. Arterial segments were isolated and mounted in vitro in a myograph, allowing the measurement of the arterial wall force.22,23 Potentiation by endothelin-1 or angiotensin II of phenylephrine-induced tone and phenylephrine-induced contractions was studied as previously described.4,22,23

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Methods

Experimental Protocols
In 137 patients undergoing coronary artery bypass surgery, excess material of internal mammary artery was used for in vitro analysis in a myograph. As previously described, a maximum of 7 to 8 segments of artery were mounted between 2 stainless steel wires in 5-mL organ baths.

Concentration-response curves to phenylephrine were performed, preceded by the determination of the response to K⁺ (125 mmol/L). At the end of the experiment, ring segments of arteries were blotted dry and weighed.

In 3 arterial segments, 2 concentration-response curves to phenylephrine (0.1 to 10 μmol/L) were performed. The second concentration-response curve to phenylephrine was made after treatment of the tissues with exogenous endothelin-1 (10 pmol/L), angiotensin II (10 pmol/L), or the solvent (control group) for 20 minutes. Changes in response to phenylephrine after addition of endothelin-1, angiotensin II, or the solvent were estimated by comparing the second phenylephrine-concentration-response curve to the first concentration-response curve. For each concentration of phenylephrine, the difference was calculated as percentage change caused by endothelin-1. In the control arterial segment, the change in tension was considered as a time control.

In 2 other ring segments, the response to contractile concentrations of endothelin-1 and calcium was tested. Contraction to calcium was tested by adding cumulative concentrations of calcium in a calcium-free depolarizing solution containing KCl (125 mmol/L). Data are expressed as a percentage of the maximum response obtained with KCl (125 mmol/L).

Endothelium-dependent (acetylcholine) and -independent (sodium nitroprusside) relaxations were tested after precontraction of the arterial segments with a concentration of phenylephrine (1 to 3 μmol/L) sufficient to produce a contraction equivalent to 50% of the maximal response. Data were expressed as a percentage dilation of phenylephrine-induced preconstriction.

Only ring segments of mammary artery responding to phenylephrine (10 μmol/L) by a contraction >5 mN and to acetylcholine (10 μmol/L) by a dilation >50% were included in the study. Similarly, a patient could be included in the study only if at least 3 segments of mammary artery were valid simultaneously. Of the original 137 patients, 48 were included in the present study and 89 were rejected. There was no significant difference in the repartition of the GG and GT/TT genotypes between the patients included and the patients excluded from the study.

Determination of the Genotype
With an approval by an ethics committee and after an informed patient consent, genomic DNA was prepared from frozen peripheral blood using standard procedures. The preproendothelin-1 fragment containing the G-to-T transversion was amplified on the basis of polymerase-chain-reaction amplification with a Perkin Elmer DNA thermal cycler and Thermus Aquaticus DNA polymerase (Boehringer). The information needed for genotyping is available on the CANVAS Web site (http://genecanvas.idf.inserm.fr/poly-morphism.asp?pol=ENDO1_5.htm).

Statistical Analysis
Results are expressed as mean±SEM. Comparison between groups was made using a 1-way ANOVA followed by a Dunnett’s test or by 2-way ANOVA for repeated measures to compare the concentration-response curves. A probability level of P<0.05 was considered significant.

Drugs
Drugs were purchased from Sigma Co.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Preoperative Characteristics of the Patients Included in the Study

<table>
<thead>
<tr>
<th>Variables</th>
<th>GG (n=21)</th>
<th>GT/TT (n=27)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>64.9±2.6</td>
<td>66.8±4.1</td>
<td>NS</td>
</tr>
<tr>
<td>Gender, m/f</td>
<td>15/6</td>
<td>17/10</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
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<td>27.9±0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Mean blood pressure, mm Hg</td>
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<td>106.2±4.1</td>
<td>NS</td>
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<td>Pharmacological therapy</td>
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<td></td>
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<tr>
<td>β-blockers</td>
<td>10</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>4</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium entry blockers</td>
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<td>10</td>
<td>NS</td>
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<td>Nitrates</td>
<td>12</td>
<td>14</td>
<td>NS</td>
</tr>
<tr>
<td>Normal left ventricle function</td>
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<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>6</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>6</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes</td>
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<td>6</td>
<td>NS</td>
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<tr>
<td>Total cholesterol, mmol/L</td>
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<tr>
<td>HDL cholesterol, mmol/L</td>
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<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
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<td>3.6±0.2</td>
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</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.4±0.3</td>
<td>1.7±0.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are n or mean±SEM. Patients with the GT/TT genotype were compared with the patients with the GG genotype using the χ² test.

Results

Patients:
Among the 48 patients included in the study, 27 had the GT/TT genotype (including 2 with TT) and 21 had the GG genotype. There was no significant difference between the 2 groups in regards to the age, gender, pathology, and pharmacological treatments (Table).

Isolated Internal Mammary Arteries
In mammary artery ring segments, the contractile response to K⁺ (125 mmol/L) was not significantly changed by the genotype (4.13±0.89 mN/mg, n=21, versus 4.71±0.55 mN/mg, n=27, GG versus GT/TT respectively).

Phenylephrine (0.1 to 100 μmol/L) induced a concentration-dependent contraction of mammary artery ring segments. The response to phenylephrine (0.1 to 100 μmol/L) was not significantly different in arterial rings from patients with the GG genotype compared with patients with the GT/TT genotype (Figure 1).

Similarly, endothelin-1 (0.1 to 300 nmol/L) induced a concentration-dependent contraction of the arterial segments that was not affected by the genotype (Figure 1).

Endothelin-1 (10 pmol/L) produced a significant potentiation of phenylephrine-induced tone (Figure 2). The expression of this amplification as a percentage of the previous response to phenylephrine showed that endothelin-1 (10 pmol/L) preferentially potentiated the contraction to phenylephrine concentrations ranging from 0.1 to 3 μmol/L (Figure 2). Endothelin-1–induced (10 pmol/L) potentiation of phenylephrine-induced (0.1 to 100 μmol/L) contractions was
significantly higher in mammary artery ring segments from patients with the GT/TT genotype compared with patients with the GG genotype (Figure 3). Similarly, angiotensin II (10 pmol/L) potentiation of phenylephrine-induced tone was significantly higher in arteries from patients with the GT/TT genotype (Figure 3). ANOVA for repeated measures was performed on the potentiation curves to either endothelin-1 or angiotensin II to confirm that there was no impact of conventional risk factors presented in Table 1 (increased body mass index, diabetes, hypertension, altered left ventricular function or dyslipemia) on the potentiation phenomenon induced by endothelin 1 or angiotensin II (data not shown). In arterial segments depolarized with a calcium-free solution of KCl (125 mmol/L), addition of exogenous calcium induced a contraction in all artery rings of both groups of patients. This contraction to calcium was higher in artery rings from patients with the GT/TT genotype (Figure 3).

Acetylcholine and sodium nitroprusside induced a concentration-dependent dilation of phenylephrine-preconstricted rings of internal mammary artery. The genotype had no significant effect on acetylcholine- or sodium nitroprusside-induced dilations (Figure 4).

**Figure 1.** Top, Concentration-response curves for phenylephrine in human internal mammary artery ring segments from patients with the GG (n=12) or GT/TT (n=16) genotype. Values are mean±SEM. Bottom, Concentration-response curves for endothelin-1 in human internal mammary artery ring segments from patients with the GG (n=12) or GT/TT (n=13) genotype. Values are mean±SEM.

**Figure 2.** Concentration-response curves for phenylephrine in human internal mammary artery ring segments (n=28) and effect of a subthreshold concentration of endothelin-1 (10 pmol/L). Bottom, The effect of endothelin-1 on the concentration-response curves for phenylephrine is expressed as percentage of control, indicating that the potentiating effect of endothelin-1 is higher for lower concentrations of phenylephrine. Values are mean±SEM.

**Figure 3.** Top, Effect of the GG (n=12) and GT/TT (n=16) genotype on endothelin-1-induced (left) or angiotensin II–induced (right) potentiation of the response to phenylephrine. Values are % of potentiation. Artery segments were obtained from patients with the GG or GT/TT genotype. Values are mean±SEM. *P<0.05, 1-way ANOVA, GG vs GT/TT. Bottom, Concentration-response curves for calcium in human internal mammary artery ring segments from patients with the GG (n=12) or GT/TT (n=13) genotype. Values are mean±SEM. *P<0.05, 2-way ANOVA, GG vs GT/TT.

**Figure 4.** Concentration-response curves for sodium nitroprusside (top) and acetylcholine (bottom) in human internal mammary artery ring segments from patients with the GG (n=12) or GT/TT (n=13) genotype. Values are mean±SEM.
Discussion

This study provides the first evidence that the gene polymorphism of preproendothelin-1 is associated with a change in vascular reactivity. Presence of the T allele was associated with an increased potentiating effect of a subthreshold concentration of endothelin-1 or angiotensin II, and with an increased responsiveness to calcium in human mammary artery ring segments from patients with coronary artery disease.

We found that a subcontractile concentration of endothelin-1 or angiotensin II potentiated the contractile response to phenylephrine in vitro. This is in agreement with previous studies that have demonstrated the occurrence of the phenomenon in vitro\textsuperscript{5–18,22,23} or in vivo,\textsuperscript{14,19–21} as well as in humans.\textsuperscript{14,20–23} We have previously shown that this potentiation involves a sensitization of the contractile apparatus to calcium through the activation of protein kinase C.\textsuperscript{15,17} Furthermore, we and others have also previously reported that endogenous endothelin-1\textsuperscript{25} and angiotensin II\textsuperscript{19–21} take part in the control of vascular tone in resistance arteries and that the concentrations of hormones used in the present study are compatible with endogenous concentrations. Hence, role of endothelin-1 in the control of vascular tone can be altered, considering that vascular levels of endothelin-1 are modified in pathological situations such as hypertension.\textsuperscript{26,27} Several epidemiological studies of the preproendothelin-1 gene polymorphism are currently being conducted; among them, one study has shown that the presence of the T allele was associated with a higher blood pressure in overweight patients.\textsuperscript{1} In the present study, we found a higher potentiating effect of both endothelin-1 and angiotensin II in mammary artery ring segments isolated from patients with the T allele.

To exclude other mechanisms that could have modulated this potentiation phenomenon, we have tested several vascular phenotypes such as direct contraction to phenylephrine and endothelin-1, contraction to K\textsuperscript{+} (125 mmol/L), and endothelium-dependent and -independent relaxations. The absence of effect of the genotype on the direct contraction to endothelin-1 (concentrations >100 pmol) and on phenylephrine-induced contraction in the absence of endothelin-1 strengthens the concept of synergy of the different systems playing a role in the control of vascular tone. Indeed, concentrations of endothelin-1 in the picomolar range are able to amplify the adrenergic-dependent tone through a reversible mechanism that does not change the amount of calcium required for the contraction.\textsuperscript{15} In addition, as high K\textsuperscript{+}-induced contractions were similar in both groups, an alteration in the contractile apparatus should be excluded. We also found that both endothelium-dependent and -independent relaxations were unaffected by the mutation. Thus, the change in vascular reactivity found can probably not be attributed to a change in endothelial function.

Therefore, potentiation of phenylephrine-induced tone by endothelin-1 might be affected. This phenomenon may be physiologically more relevant than the irreversible contraction induced by higher concentrations of endothelin-1. That both angiotensin II– and endothelin-1–induced potentiation were increased may reflect that the 2 pathways are closely related. Angiotensin II and endothelin-1 may either use the same pathway to induce a potentiation through an increased sensitivity from the contractile apparatus to calcium. This possibility is supported by the increased contractility to calcium found in arteries from patients with the T allele and by a previous observation that angiotensin II–induced tone may be blocked by endothelin receptor antagonists.\textsuperscript{28} We can also speculate that the preproendothelin-1 gene polymorphism is in linkage disequilibrium with another gene involved in the potentiation phenomenon. This could explain the impact of this polymorphism on endothelin-1 and angiotensin II control of vascular tone.

We conclude that the gene polymorphism of the preproendothelin-1 gene was associated with a change in vascular reactivity. These findings might be of interest in the interpretation of epidemiological studies and potentially in the understanding of cardiovascular diseases.

References


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