Increased Activity of Endogenous Endothelin in Patients With Type II Diabetes Mellitus

Carmine Cardillo, MD*; Umberto Campia, MD*; Melissa B. Bryant, RN; Julio A. Panza, MD

Background—Endothelial dysfunction may contribute to the risk of premature atherosclerosis in patients with diabetes. Endothelin (ET-1) may be involved in this process by activating smooth muscle cell mitogenesis and leukocyte adhesion. We sought to assess the activity of endogenous ET-1 in a group of patients with type II diabetes mellitus with the use of antagonists of ET-1 receptors.

Methods and Results—Forearm blood flow (FBF) responses (strain gauge plethysmography) to intraarterial infusion of a selective blocker of ET\textsubscript{A} receptors (BQ-123) and, on a different occasion, to ET-1, were measured in 15 patients with diabetes and 12 healthy controls. In addition, 5 patients with diabetes received coinfusion of BQ-123 and BQ-788 (a selective blocker of ET\textsubscript{B} receptors). In normal subjects, BQ-123 did not significantly modify FBF from baseline \((P=0.16)\). In contrast, BQ-123 administration resulted in a significant vasodilator response in patients with diabetes \((P<0.001)\). Infusion of exogenous ET-1 resulted in lower vasoconstrictor responses in patients with diabetes than in controls \((P=0.001)\), whereas the vasoconstrictor response to norepinephrine was similar in the 2 groups \((P=0.78)\). In patients with diabetes, the vasodilator response to selective ET\textsubscript{A} blockade was not significantly modified by nonselective blockade of ET-1 receptors obtained by coinfusion of BQ-123 and BQ-788.

Conclusions—The activity of endogenous ET-1 on ET\textsubscript{A} receptors is enhanced in the resistance vessels of patients with diabetes, whereas their sensitivity to exogenous ET-1 is blunted. This abnormality may participate in the pathophysiology of vascular complications associated with diabetes. (Circulation. 2002;106:1783-1787.)

Key Words: endothelin ■ diabetes ■ vasculature ■ receptors ■ atherosclerosis

The endothelium plays an important role in vascular homeostasis by synthesizing and releasing substances that modulate vascular tone and structure, as well as the interactions of circulating cells with the vessel wall. Patients with type II diabetes mellitus have endothelial dysfunction, defined as decreased nitric oxide–dependent vasodilator responsiveness to acetylcholine, \(^1\) which may contribute to their risk of premature atherosclerosis.

A substance potentially involved in the vasomotor deregulation of patients with diabetes, as well as in the development of their vascular complications, is endothelin (ET-1). \(^2,3\) ET-1 is a peptide that exerts its vascular effects via specific binding to 2 receptor subtypes, ET\textsubscript{A} and ET\textsubscript{B}. On vascular smooth muscle cells, both ET\textsubscript{A} and ET\textsubscript{B} receptors mediate vasoconstriction. \(^4,5\) whereas ET\textsubscript{B} receptors on endothelial cells cause vasodilation, predominantly because of activation of the L-arginine–nitric oxide pathway. \(^6\) Other effects of ET-1 include the activation of smooth muscle cell mitogenesis, leukocyte adhesion, and monocyte chemotaxis, thereby implying a potential involvement of this peptide in the initiation and/or the progression of the atherosclerotic process.

Evidence of ET-1 activation in diabetes comes from studies in both experimental models and humans. Thus, increased plasma immunoreactive ET-1 has been demonstrated in some animal models of diabetes. \(^7–9\) Similarly, elevated plasma ET-1 levels have been reported in patients with diabetes, \(^10,11\) a finding not confirmed by other reports. \(^12\) The latter discrepancy might be related to the fact that ET-1 plasma levels may not truly reflect the activity of the ET-1 system because its secretion is largely polarized toward the underlying vascular smooth muscle. \(^13\) Moreover, alterations in plasma ET-1 also may reflect changes in renal-, receptor-, or enzyme-mediated clearance of the peptide. Consequently, plasma ET-1 levels may not reflect its production or biological effects on smooth muscle cells. Recently, selective and nonselective blockers of ET-1 receptors have become available for clinical studies. This has provided a more suitable tool to assess the role of ET-1 in vascular homeostasis.

The present study, therefore, was designed to test the hypothesis of increased vasoconstrictor activity of ET-1 in the forearm resistance vessels of patients with diabetes by use of blockers of ET-1 receptors.
Clinical Characteristics of the Study Subjects

<table>
<thead>
<tr>
<th></th>
<th>Normal Controls</th>
<th>Patients With Diabetes</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female</td>
<td>5/7</td>
<td>7/8</td>
<td>0.84</td>
</tr>
<tr>
<td>Age, y</td>
<td>48±2</td>
<td>50±3</td>
<td>0.50</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>76±3</td>
<td>82±3</td>
<td>0.13</td>
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<tr>
<td>Height, cm</td>
<td>173±6</td>
<td>167±8</td>
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</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>74±3</td>
<td>78±2</td>
<td>0.25</td>
</tr>
<tr>
<td>FBF, mL/min per dL</td>
<td>2.8±0.2</td>
<td>2.6±0.2</td>
<td>0.66</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>93±3</td>
<td>157±31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma insulin, mU/mL</td>
<td>4±0.6</td>
<td>10.3±1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin A(_{1c}), %</td>
<td>...</td>
<td>7.5±0.2</td>
<td>...</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>174±8</td>
<td>183±5</td>
<td>0.32</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>110±8</td>
<td>120±5</td>
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<tr>
<td>HDL cholesterol, mg/dL</td>
<td>55±3</td>
<td>55±3</td>
<td>0.87</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>90±14</td>
<td>157±31</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

Methods

Study Subjects
Fifteen patients (Table) with a well-documented history of type II diabetes, established according to the Guidelines of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, were recruited for the present study. The time since the initial diagnosis of the disease was 42±12 months. Five patients were treated with diet alone and 10 were taking oral hypoglycemic agents. Of these 10 patients, 6 were on monotherapy with metformin (4 patients), a sulfonylurea (1 patient), or a thiazolidinedione (1 patient), and 4 were on a combination therapy of metformin and a sulfonylurea. Patients were not taking any other medication and had no clinical evidence of vascular disease, nephropathy, or retinopathy. Hypoglycemic drugs were discontinued ≥1 week before the study, and during that time, fasting glucose levels were monitored daily by glucometer. If fasting glucose levels exceeded 300 mg/dL, patients were withdrawn from the study and appropriate therapy was resumed. None of the patients had a history of hypercholesterolemia (total cholesterol >220 mg/dL), hypertension (blood pressure >140/90 mm Hg), peripheral vascular disease, coagulopathy, or any disease predisposing them to vasculitis or Raynaud’s phenomenon.

Twelve normal volunteers matched with the patients for approximate race, sex, and age, were selected as a control group (Table). Each subject was screened for clinical history, and given a physical examination, ECG, chest x-ray, and routine chemical analyses. None had evidence of present or past hypertension, hyperlipidemia, cardiovascular disease, or any other systemic condition, and none were taking medications at the time of the study. None of the subjects and patients participating in the present study was a smoker.

The National Heart, Lung, and Blood Institute Investigational Review Board approved the study protocol, and all participants gave written informed consent.

Protocols
Studies were performed in the morning in a quiet room with a temperature of ~22°C. Participants were asked to refrain from drinking alcohol or beverages containing caffeine for ≥24 hours before studies. Each study consisted of the infusion of drugs into the brachial artery and measurement of the response of the forearm vasculature by means of strain-gauge venous occlusion plethysmography, following a methodology previously described in detail. Blood pressure was recorded directly from the intraarterial catheter and heart rate was recorded from an electrocardiographic lead.

All drugs were approved for human use by the Food and Drug Administration in the form of Investigational New Drug (IND) and were prepared by the Pharmaceutical Development Service of the National Institutes of Health following specific procedures to ensure accurate bioavailability and sterility of the solutions.

Because of the prolonged infusion time required to assess the hemodynamic effect of the different substances and their relatively long-lasting effects, different studies were performed in each patient on separate days, ≥1 week apart, and in random sequence. Throughout all studies, volumes infused were matched by administration of variable amounts of saline. Given the limitations of circulating ET-1 in accurately reflecting vascular activity of the peptide, measurement of plasma ET-1 levels was not part of the present study.

Assessment of Vascular Responses to ET\(_A\) Receptor Blockade in Normal Subjects and Patients With Diabetes

Basal measurements were obtained after a 15-minute infusion of saline at 1 mL/min. Then, normal subjects and patients with diabetes received intraarterial infusion of BQ-123. BQ-123 (Peninsula Laboratories) is a synthetic peptide with high potency of antagonism for the ET\(_A\) receptor and was infused at 100 nmol/min (100-nmol/mL solution), a dose that allows it to effectively counteract the vasoconstrictor effect of endothelin-1 infusion in the human forearm. BQ-123 was given for 60 minutes (1-mL/min infusion rate), and FBF was measured every 10 minutes.

Comparison of Vascular Responses to Selective ET\(_A\) Blockade and Noneselective ET\(_A\)/ET\(_B\) Blockade in Diabetes

In 5 of the 15 patients with diabetes, the infusion of BQ-123 was extended for another 60 minutes (total infusion time: 120 minutes) at the same doses and at the same infusion rates as before, and FBF was measured every 10 minutes. On a different occasion, the same patients, after intraarterial infusion of BQ-123 for 60 minutes, received coinfusion of BQ-123 and BQ-788. BQ-788 (Peninsula Laboratories; 50-nmol/mL solution) is a synthetic and highly selective antagonist of ET\(_A\) receptors and was given at 50 nmol/min (1-mL/min infusion rate), a dose that allows a local concentration in the forearm >10-fold higher than the pA\(_2\) at the ET\(_A\) receptor. The combination of BQ-123 and BQ-788 was infused for 60 minutes and FBF was measured every 10 minutes.

Statistical Analysis
Two means were compared by paired or unpaired Student’s t test, as appropriate. Within each group, changes in FBF from baseline in response to each drug were assessed by one-way ANOVA for repeated measures. Comparisons in the responses to ET-1 receptor blockade, norepinephrine, and ET-1 between the two groups were
performed with the use of two-way ANOVA, followed by Bonferroni t test for pairwise comparisons. Comparison of the effect of selective ET A blockade versus combined ET A/B blockade in patients with diabetes was performed by two-way ANOVA for repeated measures. Multiple comparisons were performed with the use of Dunnett’s test. All calculated probability values are two-tailed, and a probability value, $P < 0.05$ was considered to indicate statistical significance. All group data are reported as mean±SEM.

Results

Mean arterial pressure and heart rate did not significantly change after infusion of any of the drugs used in the study, thus indicating that the drug effects were limited to the infused forearm. Baseline FBF was similar in patients with diabetes and healthy controls at all occasions (all $P > 0.05$).

Vascular Responses to ET A Receptor Blockade in Normal Subjects and Patients With Diabetes

In control subjects, infusion of BQ-123 did not significantly modify FBF from baseline ($P = 0.16$). In contrast, in patients with diabetes, BQ-123 administration resulted in a significant vasodilator response ($P < 0.001$ versus baseline). As a result, FBF values during selective ET A blockade were significantly higher in patients with diabetes than in controls (Figure 1).

Vascular Responses to Endothelin-1 and Norepinephrine in Normal Subjects and Patients With Diabetes

ET-1 caused a significant vasoconstrictor response in both patients and controls (both $P < 0.001$ versus baseline), but this effect was significantly blunted in patients with diabetes compared with controls (Figure 2).

The infusion of increasing doses of norepinephrine induced a progressive vasoconstrictor response in both patients with diabetes and controls. In contrast with the results of ET-1 infusion, during infusion of norepinephrine at 60, 120, and 240 pmol/min, FBF was reduced by 18%, 24%, and 27%, respectively, in normal subjects ($P < 0.001$ versus baseline) and by 17%, 24%, and 26% in patients with diabetes ($P < 0.001$ versus baseline), without any significant difference between the 2 groups ($P = 0.78$).

Vascular Responses to Selective ET A Versus Nonselective ET A and ET B Blockade in Patients With Diabetes

In patients with diabetes, the magnitude of the vasodilator response from baseline during the initial 60 minutes of BQ-123 administration was not different between the 2 occasions (Figure 3, left). Prolongation of BQ-123 for 2 hours did not result in any significant change in the degree of the vasodilator response compared with that observed after 60 minutes ($P = 0.74$). Similarly, superimposing of BQ-788 to BQ-123 did not significantly modify the vasodilation induced by BQ-123 induced alone ($P = 0.92$). As a result, FBF values were not significantly different during selective ET A than during nonselective ET-1 blockade (Figure 3, right).

Discussion

The present study demonstrates that blockade of ET A receptors results in vasodilation in patients with diabetes but not in controls, thereby suggesting that ET A-dependent vasoconstrictor activity is enhanced in diabetes.

Different mechanisms may explain this increased ET A-dependent vasoconstriction in diabetes, such as increased availability of ET-1 at the ET A receptor level or enhanced susceptibility of blood vessels to the vasoconstrictor effects of ET-1. To better define which mechanism is effective in diabetic vessels, we compared vascular responsiveness to administration of exogenous ET-1 in patients and controls. Our results indicate that the vasoconstrictor effect of ET-1 is blunted in patients with diabetes compared with controls. This phenomenon is unlikely to be explained by nonspecific reduction of vascular reactivity to vasoconstrictor stimuli in
diabetic vessels, because the response to norepinephrine was similar in the 2 groups. Therefore, these results suggest that an increased production of ET-1 is a more likely mechanism to explain the enhanced ET_{A}-dependent vasoconstrictor activity observed in diabetes. It must be noted, however, that our methodology does not allow direct assessment of vascular ET-1 levels and, therefore, it is not possible to quantify the magnitude of ET-1 overproduction in diabetic vessels.

Both hyperinsulinemia and hyperglycemia are potential causes of increased production of ET-1 in patients with diabetes. Thus, insulin has been shown to increase ET-1 gene expression in cultured endothelial cells \(^{18}\) and to enhance ET-1 release in both human endothelial and vascular smooth muscle cells \(^{19,20}\). Also, it has been demonstrated in humans that hyperinsulinemia is associated with increased plasma ET-1 levels \(^{19,21}\). Moreover, previous studies in our laboratory have demonstrated that insulin infusion in the forearm circulation of healthy subjects induces a pattern of activation of the ET-1 system similar to that observed in the present study in diabetics \(^{22}\). Similarly, a possible role of hyperglycemia in stimulating ET-1 production is suggested by studies showing that ET-1 secretion is enhanced in cultured aortic endothelial cells exposed to elevated glucose levels \(^{23}\). Because the present study was not specifically designed for this purpose, it does not allow direct conclusions about the relative contribution of hyperinsulinemia and hyperglycemia to the increased vascular activity of ET-1 observed in patients with diabetes.

In contrast to the results obtained in patients with diabetes, selective blockade of ET_{A} receptors did not result in any significant change of vascular tone in normal subjects. These findings are in keeping with those previously observed in our laboratory \(^{24,25}\), but are at odds with those observed by other investigators who infused BQ-123 in normal subjects \(^{6,16,26}\). Because all previous studies used similar methodology, there is no clear explanation for this discrepancy. However, our conclusions of increased ET-1 vasoconstrictor activity in patients with diabetes are based on the striking difference in the response to ET-1 receptor blockers between healthy controls and patients with diabetes studied in the same laboratory. Therefore, discrepancies in the response of normal subjects between this and previous investigations should not affect the interpretation of our study findings.

In the present study, reduced vasoconstrictor responsiveness to exogenous ET-1 was observed in patients with diabetes compared with controls. This finding is in keeping with those of previous studies reporting decreased responsiveness to ET-1 in both aortic rings \(^{24,27}\) and perfused mesenteric arterial beds \(^{8}\) of streptozotocin rats. Importantly, the same phenomenon has also been recently reported in human small resistance vessels from subcutaneous biopsies in vitro \(^{28}\). Similarly, blunted vasoconstrictor response to ET-1 despite preserved vascular smooth muscle function has been observed in the forearm of patients with diabetes \(^{29}\). Although changes in endothelial or smooth muscle ET_{A} receptor activity in patients with diabetes might have contributed to their blunted responsiveness to exogenous ET-1, it must be noted that the hemodynamic effect of ET-1, at the doses given in the present study, is entirely dependent on its action on ET_{A} receptors \(^{16}\). Therefore, the attenuation of vasoconstrictor responsiveness to ET-1 observed in our patients with diabetes is likely related to downregulation of ET_{A} receptors resulting from increased production of the peptide.

In a subgroup of the patients with diabetes included in the present study, the vasodilator response to selective ET_{A} blockade was not modified by the addition of the ET_{B} receptor antagonist. Experiments with ET_{A/B} receptor blockade were performed mainly to assess whether, from a therapeutic standpoint, nonselective ET_{A/B} blockade could provide some additional hemodynamic benefit over selective ET_{A} blockade. In this regard, our observation that, in patients with diabetes, vasodilation is similar during BQ-123 alone and the combination of BQ-123 and BQ-788 substantiates the conclusion that nonselective ET_{A/B} receptor blockade does not offer any hemodynamic advantage over selective ET_{A} blockade. This seems consistent with the notion of “neutral” overall contribution of ET_{B} receptors (endothelial and smooth muscle), implying either that both ET_{B} receptors are normally functioning in patients with diabetes or that they undergo consensual changes (up- or downregulation) in these patients.

This latter finding is at odds with those previously observed in our laboratory in other patient groups and underscores the specificity of the changes in vascular ET-1 activity in patients with diabetes. Thus, in the human resistance arteries of hypercholesterolemic patients, ET_{B} receptor antagonism blunts the vasodilator response induced by selective ET_{A} blockade, suggesting that vasodilation is the predominant hemodynamic effect of ET_{B} receptor stimulation \(^{25}\). Also, in patients with essential hypertension, nonselective ET_{A/B} receptor antagonism results in greater enhancement of the vasodilator response to selective ET_{A} blockade, suggesting impaired ET_{A/B}-mediated vasodilation in these patients \(^{24}\). Furthermore, the differences in vascular activity of the ET-1 system between patients with diabetes and those with other risk factors for cardiovascular disease are emphasized.
by the discrepancies observed in the responsiveness to exogenous ET-1. Thus, previous studies in our laboratory have shown that vasoconstrictor responsiveness to infused ET-1 is not reduced in other conditions associated with increased production of endogenous ET-1, such as arterial hypertension and hypercholesterolemia. In conjunction, these observations suggest that, in addition to downregulation of ET\(_A\) receptors, other mechanisms, such as the pathological changes known to occur in diabetic blood vessels over time, may importantly contribute to the blunted vasoconstrictor effect of ET-1 in patients with diabetes.

Several observations suggest a potential role of activated ET-1 system in the pathophysiology of complications of diabetes mellitus. Thus, hypertension, which occurs more in patients with diabetes than in the general population, may be related to increased ET-1 activity, as supported by recent studies. Similarly, the higher incidence of atherosclerotic vascular disease in patients with diabetes may also be related to the atherogenic properties of ET-1. Finally, an involvement of ET-1 might be postulated in other complications of diabetes, such as neuropathy, retinopathy and nephropathy.

The results of the present study may have important clinical implications. Our demonstration that ET-1–dependent vasoconstrictor tone is enhanced in the blood vessels of patients with diabetes not only indicates an involvement of this peptide in the pathophysiology of organ damage, but also suggests that targeting the ET-1 system might be potentially beneficial in preventing or treating cardiovascular disease in diabetes. In this regard, our findings suggest that selective ET\(_A\) blockade and nonselective ET\(_{AB}\) blockade would be equally effective in preserving vascular homeostasis in diabetes.

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References
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