Enhanced Vascular Activity of Endogenous Endothelin-1 in Obese Hypertensive Patients

Carmine Cardillo, Umberto Campia, Micaela Iantorno, Julio A. Panza

Abstract—Hypertensive patients have increased endothelin-1–dependent vasoconstrictor tone. This abnormality, however, might not be uniformly present in all forms of hypertension, as suggested by experimental studies showing that endothelin-1 activity is enhanced predominantly in low-renin, high-volume models and in insulin-resistant states. Because hypertension in obesity is commonly associated with both expanded plasma volume and insulin resistance, this study sought to determine whether increased body mass index (BMI) in hypertensive patients relates to activation of the endothelin-1 system. Forearm blood flow (FBF) responses (plethysmography) to intra-arterial infusion of an ETα receptor blocker (BQ-123) were analyzed in hypertensive patients and normotensive control subjects according to BMI. The vasodilator response to BQ-123 was significantly higher in hypertensive patients than in control subjects (P<0.001). During BQ-123, a significant increase in FBF from baseline was observed in obese (BMI ≥30 kg/m²; P<0.001) and overweight (BMI, 27 to 29.9 kg/m²; P=0.04) but not in lean (BMI <27 kg/m²; P=0.83) hypertensive patients. In contrast, no significant change in FBF was observed during BQ-123 either in obese (P=0.53), overweight (P=0.76), or lean (P=0.93) normotensive subjects. Moreover, a significant correlation between BMI and the vasodilator response to ETα blockade was observed in hypertensive subjects (R=0.53; P=0.005) but not in control subjects (R=0.11; P=0.58). In human hypertension, increased BMI is associated with enhanced ETα-dependent vasoconstrictor activity, suggesting that this abnormality may play a role in the pathophysiology of obesity-related hypertension and that targeting the endothelin-1 system may be useful in the treatment of these patients. (Hypertension. 2004;43:36-40.)

Key Words: endothelin ■ hypertension, obesity ■ vasculature ■ atherosclerosis

Previous work in our laboratory with infusion of endothelin (ET) receptor antagonists in the human forearm circulation demonstrated that ET-1–dependent vasoconstrictor tone is increased in patients with essential hypertension and may play a role in the pathophysiology of hypertension–related vascular damage.1 Also, blockade of ET-1 receptors in patients with hypertension enhances endothelin-dependent vasodilation,2 thereby suggesting that activation of the ET-1 system may contribute to the atherosclerotic process in hypertensive vessels.

Because essential hypertension is a heterogeneous condition, an activation of the ET-1 system might not be a generalized finding in all patients. This view is supported by the results of previous studies in animal models, indicating that ET-1 activity is predominantly enhanced in low-renin, high-volume forms of experimental hypertension.3 Thus, in DOCA-salt hypertensive rats, ET-1 gene expression is enhanced and abolishment of ET-1 overexpression results in lower blood pressure with regression of vascular growth.4,5 Similar results have been observed in Dahl salt-sensitive rats, another model of low-renin, high-volume hypertension.6 Moreover, increased renal ET-1 mRNA expression, in conjunction with ETα/ETβ receptor imbalance, differentiates salt-sensitive from salt-resistant forms of spontaneous hypertension.7 Finally, experiments in transgenic rats with tissue-selective disruption of ETβ receptors have recently shown that these animals have extreme salt-sensitive hypertension as a consequence of abnormally high sodium reabsorption in the collecting duct, thereby emphasizing the importance of ET-1 in the pathogenesis of hypertension associated with expanded plasma volume.8

Another condition often associated with activation of the ET-1 system is insulin resistance. Thus, studies in insulin-resistant animals have clearly shown an activation of the ET-1 system in these models, also indicating a role of ET-1 in the pathogenesis of blood vessel dysfunction in this condition.9,10 Since hypertension associated with obesity is commonly characterized by both plasma volume expansion and insulin resistance,11 it is reasonable to postulate that a particularly enhanced vasoconstrictor activity may exist in those patients and play a part in the pathophysiology of the hypertensive process and its complications in obesity. This has also been suggested by recent studies showing a correlation between ET-1 gene polymorphism and blood pressure levels in obese Japanese subjects.12,13

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Clinical Characteristics of the Study Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal Control Subjects (n=28)</th>
<th>Hypertensive Patients (n=27)</th>
<th>P</th>
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<td>15/12</td>
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<td>Age, y</td>
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<td>50±1</td>
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<td>Weight, kg</td>
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<td>Height, cm</td>
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<td>170±2</td>
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<td>&lt;0.001</td>
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<td>Fasting glucose</td>
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<td>mmol/L</td>
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<td>mg/dL</td>
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<td>Total cholesterol</td>
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<td>mg/dL</td>
<td>99±12</td>
<td>110±14</td>
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</tbody>
</table>

Data are expressed as mean±SEM. MAP indicates mean arterial pressure; LDL, low-density lipoprotein; and HDL, high-density lipoprotein.

The current study, therefore, was designed to investigate whether enhanced ET-1 activity in hypertensive patients is related to increased body mass. We also assessed whether differences exist in the activity of the ET-1 system between obese normotensive and hypertensive subjects.

Methods

Study Subjects

The population included 27 hypertensive patients and 28 healthy control subjects who participated in prospective studies conducted in the Cardiology Branch of the National Heart, Lung, and Blood Institute (NHLBI), designed to investigate the in vivo vasoconstrictor activity of ET-1 in hypertension (Table). Hypertensive patients were followed at the outpatient clinic and had a well-documented history of chronically elevated blood pressure (≥140/90 mm Hg) without any apparent underlying cause. Patients were not taking any other medication, and antihypertensive drugs were discontinued at least 2 weeks before the study. None of the patients had a history of hypercholesterolemia, diabetes mellitus, coagulopathy, or any disease predisposing them to vasculitis or Raynaud’s phenomenon. Normal volunteers selected as a control group were matched with the patients for approximate race, gender, and age. Each subject was screened by clinical history, physical examination, ECG, chest radiography, and routine chemical analyses. None had evidence of present or past hypertension, hyperlipidemia, cardiovascular disease, or any other systemic condition, and none of them was taking medications at the time of the study. None of the subjects and patients participating in this study was a smoker.

The study protocol was approved by the Investigational Review Board of the National Heart, Lung, and Blood Institute, 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 at least 24 hours before studies. Each study consisted of infusion of drugs into the brachial artery and measurement of the response of the forearm vasculature by means of strain-gauge venous occlusion plethysmography. Blood pressure was recorded directly from the intra-arterial 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 following specific procedures to ensure accurate bioavailability and sterility of the solutions.

Throughout all studies, volumes infused were matched by administration of variable amounts of normal saline solution.

Assessment of Effects of Body Mass on Vascular Responses to ET<sub>A</sub> Receptor Blockade in Normal Subjects and Hypertensive Patients

Basal measurements were obtained after a 15-minute infusion of saline at 1 mL/min. Then, normal subjects and hypertensive patients received intra-arterial infusion of BQ-123. BQ-123 (Peninsula Laboratories) is a synthetic peptide with high potency of antagonism for the ET<sub>A</sub> receptor<sup>14</sup> and was infused at 100 nmol/min (100 nmol/mL solution), a dose that allows effective counteraction of the vasoconstrictor effect of endothelin-1 infusion in the human forearm.<sup>15</sup> BQ-123 was given for 60 minutes (1 mL/min infusion rate), and forearm blood flow (FBF) was measured every 10 minutes.

To investigate the possible association between increasing body mass and vascular responses to selective ET<sub>A</sub> blockade in normotensive and hypertensive subjects, body mass index (BMI), defined as the weight (kg) divided by the square of the height (m<sup>2</sup>), was evaluated as both a categorical and a continuous variable. For the categorical analysis, subjects and patients were divided into 3 groups: lean (BMI <27 kg/m<sup>2</sup>), overweight (BMI 27 to 29.9 kg/m<sup>2</sup>), or obese (BMI ≥30 kg/m<sup>2</sup>). Association between BMI as continuous variable with vascular responses to BQ-123 was analyzed by linear regression analysis.

Assessment of Vascular Responses to Endothelin-1 in Obese Normotensive and Hypertensive Subjects

To determine whether a difference exists in vascular sensitivity to the hemodynamic effects of ET-1 between obese normotensive and hypertensive subjects, experiments were performed on a separate day to compare the vasomotor responses to exogenous ET-1 in the two groups. To this end, after basal measurements were obtained, 6 obese normotensive subjects and 7 obese hypertensive subjects received intra-arterial infusion of ET-1. ET-1 (Bachem Inc; 5 pmol/mL solution) was given at 5 pmol/min (1 mL/min infusion rate) for 60 minutes, and FBF was measured at 10-minute intervals.

Statistical Analysis

Two means were compared by Student t test. Within each group, changes in FBF from baseline in response to the infused drugs were assessed by 1-way ANOVA for repeated measures. Group comparisons of the responses to selective ET<sub>A</sub> blockade and to exogenous ET-1 infusion were performed by 2-way ANOVA. When significant differences were found by ANOVA, post hoc analyses for multiple comparisons were performed by Dunnett or Student-Newman-Keuls test, as appropriate. Correlations were tested by linear regression analysis. All calculated probability values are 2-tailed, and a probability value of <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 not significantly different between groups at all times (all $P>0.05$).

**Effects of Body Mass on Vascular Responses to ET\textsubscript{A} Receptor Blockade in Normal Subjects and Hypertensive Patients**

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

During administration of BQ-123, a significant increase in FBF from baseline was observed in both obese ($n=11$) and overweight ($n=7$) hypertensive subjects, whereas no significant changes were observed in lean hypertensive subjects ($n=9$) (Figure 2, left panel). As a consequence, FBF during BQ-123 was significantly different among these 3 groups ($P<0.001$). Comparisons across groups by post hoc pairwise analysis demonstrated that the vasodilator response to BQ-123 was not significantly different between overweight and obese hypertensive subjects ($P>0.05$), whereas it was significantly higher in both overweight and obese subjects when each was compared with that of lean hypertensive subjects (both $P<0.05$). In contrast, with the results observed in hypertensive patients, no significant change in FBF from baseline was observed in the three groups of normotensive subjects (lean, $n=12$, $P=0.53$; overweight, $n=7$, $P=0.76$; obese, $n=9$, $P=0.93$) (Figure 2, right panel).

In hypertensive patients, a significant correlation was observed between BMI and the vasodilator effect of BQ-123 (Figure 3, left panel). No significant correlation between BMI and the response to BQ-123, in contrast, was observed in normotensive subjects (Figure 3, right panel).

**Vascular Responses to Endothelin-1 in Obese Normotensive and Hypertensive Subjects**

ET-1 caused a significant vasoconstrictor response in both obese normotensive and hypertensive subjects (both $P<0.001$ versus baseline), but this effect was not significantly different between the two groups (Figure 4).

**Discussion**

The main finding of the present study is that blockade of ET\textsubscript{A} receptors results in significant vasodilation in overweight and obese but not in lean hypertensive subjects; in contrast, it does not determine any significant hemodynamic change in normotensive control subjects, irrespective of their body mass. In our study, BQ-123–induced vasodilation was significantly related to BMI in hypertensive subjects but not in control subjects, thereby indicating a selective enhancement of ET\textsubscript{A}-dependent vasoconstrictor tone in hypertensive patients with increased body mass.

Different mechanisms may potentially explain the increased ET\textsubscript{A}-dependent vasoconstriction in hypertensive patients with increased body mass, such as increased availability of ET-1 at the ET\textsubscript{A} receptor level or enhanced susceptibility of their blood vessels to the vasoconstrictor effects of ET-1. To better define the mechanism underlying this abnormality, we compared vascular responsiveness to...
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administration of exogenous ET-1 in obese normotensive and hypertensive subjects. Our results indicate that the vasoconstrictor effect of ET-1 in obesity is independent of blood pressure levels because vascular responsiveness to exogenous ET-1 was similar in obese normotensive and hypertensive subjects. This finding argues against the possibility of enhancement of vascular reactivity to ET-1 induced by the structural changes commonly present in hypertensive vessels and suggests that an increased production of ET-1 is the most likely mechanism to explain this abnormality. It must be noted, however, that our methodology does not allow direct assessment of vascular levels of ET-1, and, therefore, it is not possible to quantify the magnitude of ET-1 overproduction in these patients.

Several potential factors may account for an increased vascular production of ET-1 in hypertensive patients with increased body mass. For example, hyperinsulinemia, a common finding in obesity, may importantly affect the activity of the ET-1 system, since insulin has been shown to increase ET-1 gene expression in cultured endothelial cells and to enhance ET-1 release in both human endothelial and vascular smooth muscle cells. Also, it has been demonstrated in humans that hyperinsulinemia is associated with increased plasma ET-1 levels. Moreover, previous studies in our laboratory using antagonists of ET-1 receptors have demonstrated that insulin infusion in the forearm circulation is able to elicit an activation of the ET-1 system. Leptin or other adipokines, such as resistin, might also play a role, since previous studies have shown that leptin upregulates ET-1 production in human endothelial cells in culture, and resistin may promote ET-1 release from cultured endothelial cells by inducing ET-1 promoter activity. Because we did not perform measurements of insulin, leptin, or resistin in our patients, we cannot determine to what extent these factors may have contributed to our findings, and further studies are therefore needed to address this issue. It is important to emphasize, however, that whatever factors are involved in this process, they seem to become effective only when obesity and hypertension are associated, therefore suggesting the possibility that activation of the ET-1 system in the setting of increased body mass may play a role in the development or maintenance of high blood pressure. In this regard, our results are in apparent contrast with those previously reported by Mather et al., who have recently reported that obesity per se is associated with enhanced ET<sub>A</sub>-mediated vasoconstrictor tone in the leg circulation and that blockade of ET<sub>A</sub> receptors reverses endothelial dysfunction in these patients. It must be noted, however, that blood pressure values in their group of obese patients were significantly higher than those of control subjects, which may have importantly contributed to their study results.

Perspectives

Several lines of reasoning suggest a potential role of the activated ET-1 system in the pathophysiology of complications of obesity-related hypertension. For example, the atherogenic properties of ET-1<sup>25</sup> may play a role in the development of the atherosclerotic vascular disease in obese hypertensive subjects. Similarly, ET-1 may be involved in other complications of hypertension in obesity, such as cardiac remodeling and heart failure<sup>26</sup> or renal damage.<sup>27</sup> The results of the present study, therefore, may have important clinical implications. Our demonstration that ET-1-dependent vasoconstrictor tone is selectively enhanced in blood vessels of hypertensive patients with increased body mass not only supports the notion of an involvement of this peptide in the pathophysiology of blood pressure elevation in these patients but also suggests that targeting the ET-1 system might be potentially beneficial in preventing or treating hypertension and its complications in obesity.

Acknowledgments

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