Excess Norepinephrine Impairs Both Endothelium-Dependent and -Independent Vasodilation in Patients With Pheochromocytoma

Yukihito Higashi, Shota Sasaki, Keigo Nakagawa, Masashi Kimura, Satoshi Sasaki, Kensuke Noma, Hideo Matsuura, Keiko Hara, Chikara Goto, Tetsuya Oshima, Kazuaki Chayama

Abstract—There is little information concerning the interaction of nitric oxide and norepinephrine (NE) on endothelial function in humans. The purpose of this study was to determine whether endothelial function is impaired by NE secreted from patients with pheochromocytoma (pheo) and whether surgical resection of the tumor improves endothelial function in these patients. We evaluated the forearm blood flow (FBF) response to acetylcholine (ACh), an endothelium-dependent vasodilator, and isosorbide dinitrate (ISDN), an endothelium-independent vasodilator, before and after adrenalectomy in 8 pheo patients, 20 normotensive subjects, and 20 patients with essential hypertension. FBF was measured using a mercury-filled silastic strain-gauge plethysmograph. The FBF response to ACh was the greatest in normotensive subjects and the least in pheo patients. The FBF response to ISDN was significantly less in pheo patients than in the other 2 groups, which had similar responses to ISDN. Adrenalectomy significantly decreased plasma and urinary NE, systolic and diastolic blood pressures, heart rate, and forearm vascular resistance. After adrenalectomy, FBF responses to both ACh and ISDN were enhanced in all pheo patients. The ratio of maximal ACh-stimulated FBF to maximal ISDN-stimulated FBF was significantly higher after adrenalectomy than before adrenalectomy (2.1 ± 0.4 versus 1.1 ± 0.1; P < 0.05). The increase in maximal FBF response to ACh correlated significantly with the decrease in urinary excretion of NE (r = −0.62, P < 0.01). These findings suggest that excess NE from pheo may predominately impair endothelium-dependent vasodilation in humans. (Hypertension. 2002;39[part 2]:513-518.)

Key Words: pheochromocytoma ■ norepinephrine ■ nitric oxide ■ acetylcholine ■ endothelial function ■ hypertension, essential

A balance of vasodilators and vasoconstrictors plays an important role in the physiological regulation of vascular tone.1,2 Nitric oxide (NO), one of the most important vasodilating agents, is released under physiological conditions in response to stimuli such as shear stress, as well as in response to many pharmacologic stimuli such as receptor agonists and circulating hormones.3–5 Endothelium-dependent vasodilation in response to receptor-dependent agonists, including acetylcholine (ACh) and bradykinin, is impaired in patients with essential hypertension through a reduced NO production.6–9 Although norepinephrine (NE) is not released from vascular endothelium, it is a major vasoconstricting factor. NE stimulates α-adrenergic receptors, causing vasoconstriction and glycolysis. Several previous studies have shown that both NO and NE contribute to the pathogenesis, development, and maintenance of hypertension.1,2,4,5,10 However, there is little information concerning the interaction of NO and NE on endothelial function in humans.

Patients with pheochromocytoma (pheo)-secreted NE are ideal models for determining how endothelium-dependent and -independent vasodilation is affected under the influence of excess vasoconstrictors. We hypothesized that endothelial function is impaired in patients with adrenal pheo secreting NE and that surgical resection of the tumor would improve endothelial function in these patients.

To determine the role of excess NE in endothelial function, we evaluated the endothelium-dependent vasodilation induced by ACh and the endothelium-independent vasodilation induced by isosorbide dinitrate (ISDN) before and after adrenalectomy in patients with adrenal pheo secreting NE as compared with normotensive subjects and essential hypertensive patients.

Methods

Study Protocol I: Endothelial Function in Normotensive Subjects, Essential Hypertensive Patients, and Pheo Patients

We studied 8 patients with adrenal pheo secreting NE (5 men and 3 women; mean age: 38 ± 11 years) and 20 normotensive subjects (14

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men and 6 women; mean age: 37±6 years) and 20 patients with essential hypertension (13 men and 7 women; mean age: 40±13 years). The study protocol was approved by the ethics committee of the Hiroshima University Faculty of Medicine. Informed consent for participation was obtained from all subjects.

**Essential Hypertensive Patients**
Hypertension was defined as a systolic blood pressure $\geq 140$ mm Hg and/or a diastolic blood pressure $\geq 90$ mm Hg measured in a sitting position on at least 3 different occasions in the outpatient clinic of Hiroshima University Faculty of Medicine. Secondary hypertensive patients were excluded on the basis of a complete history and physical examination, radiologic and ultrasonar examinations, and urinalysis. Plasma renin activity, aldosterone and NE concentrations, and serum creatinine, potassium, calcium, and free thyroxine concentrations were determined; 24-hour urinary excretion of catecholamines, 17-hydroxycorticosteroids, 17-ketogenic steroids, and vanillylmandelic acid was measured as well. None of the patients had a history of antihypertensive treatment before the study.

**Adrenal Pheo Patients**
A diagnosis of pheo secreting NE was confirmed by biologic and pharmacologic tests, including the measurement of plasma NE ($>2$ ng/mL) and 24-hour urinary excretion of NE ($>500$ μg/d) and vanillylmandelic acid (15 mg/d). Location of the tumor was identified by computed tomography, magnetic resonance imaging, and scintigraphy using an analogue of guanethidine labeled with $^{131}$I-metaiodobenzylguanidine. Only patients with a single adrenal tumor (right side: n=3; left side: n=5) were included. No patient had multiple endocrine neoplasias. No antihypertensive agents were taken by these patients for $>2$ weeks before the study.

**Normotensive Subjects**
Normal blood pressure was defined as a systolic blood pressure $<140$ mm Hg and a diastolic blood pressure $<90$ mm Hg. The normotensive control subjects had no history of serious diseases. No patients, including essential hypertensive and pheo patients, were currently smoking or had a history of smoking.

Forearm vascular responses to ACh (Daiichi Pharmaceutical Co) and ISDN (Eisai Pharmaceutical Co) were evaluated in all subjects. The study began at 8:30 AM. Subjects fasted the previous night for at least 12 hours. They were kept in the supine position in a quiet, dark, air-conditioned room (temperature 22°C to 25°C) throughout the study. A 23-gauge polyethylene catheter (Hakcow Co) was inserted into the left brachial artery for the infusion of ACh and ISDN and for the recording of arterial pressure with an AP-641G pressure transducer (Nihon Kohden Co) under local anesthesia (1% lidocaine). Another catheter was inserted into the left deep antecubital vein to obtain blood samples. After the patients were in the supine position for 30 minutes, we measured forearm blood flow (FFB) and arterial blood pressure. Then, the effects of the ACh and ISDN on forearm hemodynamics were measured. ACh (7.5, 15, and 30 μg/min) and ISDN (0.75, 1.5, and 3.0 μg/min) were infused intra-arterially for 5 minutes at each dose using a constant rate infusion pump (Terfusion STG-523, Termo Co). The FBF was measured during the last 2 minutes of the infusion. The infusions of ACh and ISDN were performed in a random order. Each study proceeded after the FBF had returned to baseline. Baseline fasting serum concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, creatinine, inulin, glucose, electrolytes, and plasma nitrate/nitrite (NOx) and NE concentrations were obtained after the 30-minute rest period.

**Study Protocol 2: Effect of Adrenalectomy on Endothelial Function in Patients With Pheo**
Vasodilatory responses to ACh and ISDN were evaluated in a manner identical to study protocol 1 before adrenalectomy and within 4 weeks after this procedure in 6 patients with pheo. We confirmed that plasma and urinary NE concentrations and blood pressures were in the normal range after 2 weeks of surgery in all subjects. Surgical approaches to adrenalectomy included the open transabdominal approach in 3 of the patients and laparoscopic adrenalectomy in the remaining 5.

**Measurement of FBF**
The FBF was measured using a mercury-filled Silastic strain-gauge plethysmograph (EC-5R, D.E. Hokanson, Inc) as previously described. FBF was expressed as milliliters per minute per 100 mL of forearm tissue volume. Four plethysmographic measurements were averaged for analysis of FBF at baseline and during administration of drugs. Forearm vascular resistance (FVR) was calculated as the mean arterial pressure divided by FBF.

**Analytical Methods**
Routine chemical methods were used to determine serum concentrations of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, creatinine, glucose, and electrolytes. Plasma concentrations of NOx were assayed by colorimetric methods using NOx assay kits (Cayman Chemical Co). The plasma and urinary concentrations of NE were measured by high-performance liquid chromatography.

**Statistical Analysis**
Results are presented as the mean±SD. Values of $P<0.05$ were considered significant. Multigroup comparisons of variables was performed by the 1-way ANOVA followed by the Bonferroni correction. Comparisons of parameters before and after adrenalectomy were performed with adjusted means by analysis of covariance using baseline data as covariates. Comparisons of time-course curves of parameters during the infusions of ACh and ISDN were analyzed by 2-way ANOVA for repeated measures on one factor followed by the Bonferroni correction for multiple-paired comparisons. When an interactive effect of drug reached statistical significance, the nature of this interaction was further investigated by applying 2-way ANOVA for repeated measured to 3 groups (normotensive, essential hypertensive, and pheo) without consideration of the factorial design. Relationships between variables were determined by linear regression analysis. The data were processed using the software packages StatView IV (SAS Institute Inc.) or Super ANOVA (Abacus Concepts).

**Results**
**Study Protocol 1: Endothelial Function in Normotensive Subjects, Patients With Essential Hypertension, and Patients With Pheo**
The baseline clinical characteristics of the 20 normotensive individuals, 20 essential hypertensive patients, and 8 patients with pheo are summarized in the Table. The systolic and diastolic blood pressures as well as FVR were significantly higher in essential hypertensive patients and patients with pheo than in normotensive subjects. These parameters were similar in essential hypertensive patients and patients with pheo. Heart rate, serum glucose, plasma, and urinary NE were significantly higher and serum insulin was significantly lower in patients with pheo compared with the other groups. The other parameters were similar between the 3 groups.

The intra-arterial infusion of ACh significantly increased FBF in a dose-dependent manner in all 3 groups. The response of FBF to ACh was greatest in normotensive subjects and least in patients with pheo (pheo versus essential hypertension, pheo versus normotension, and essential hypertension versus normotension, $P<0.0001$ for all; Figure 1, top). The intra-arterial infusion of ISDN significantly increased FBF in a dose-dependent manner in all 3 groups. The FBF response to ISDN was significantly smaller in patients with pheo than in the other groups ($P<0.001$ for all).
Clinical Characteristics of Normotensive Subjects, Hypertensive Patients, and Before and After Adrenalectomy in Patients With Pheo

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normotensive (n=20)</th>
<th>Hypertensive (n=20)</th>
<th>Pheo (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>23.3±1.8</td>
<td>23.4±1.9</td>
<td>22.1±1.7</td>
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<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>114.2±8.2</td>
<td>152.4±12.4*</td>
<td>161.4±17.5*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>67.6±6.1</td>
<td>95.8±6.9*</td>
<td>98.7±8.9*</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>68.2±6.2</td>
<td>67.4±6.4</td>
<td>79.4±9.3*</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.12±0.68</td>
<td>5.19±0.80</td>
<td>5.78±0.98</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.18±0.56</td>
<td>1.34±0.57</td>
<td>1.76±0.79</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.41±0.40</td>
<td>1.20±0.43</td>
<td>1.16±0.64</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.51±0.55</td>
<td>3.47±0.58</td>
<td>3.78±0.77</td>
</tr>
<tr>
<td>Serum glucose (mmol/dL)</td>
<td>4.8±0.4</td>
<td>5.1±0.6</td>
<td>5.8±1.2*</td>
</tr>
<tr>
<td>Serum Insulin (pmol/L)</td>
<td>54.3±9.8</td>
<td>60.1±12.4</td>
<td>42.1±15.4*</td>
</tr>
<tr>
<td>Plasma NOx (µmol/L)</td>
<td>32.8±16.3</td>
<td>29.7±18.1</td>
<td>33.1±20.2</td>
</tr>
<tr>
<td>Plasma NE (ng/mL)</td>
<td>0.23±0.18</td>
<td>0.26±0.16</td>
<td>0.71±3.28*</td>
</tr>
<tr>
<td>Urinary NE (µg/d)</td>
<td>74.3±12.3</td>
<td>80.1±13.5</td>
<td>130.6±385.2*</td>
</tr>
<tr>
<td>FBF (mL/min per 100 mL tissue)</td>
<td>4.7±1.2</td>
<td>4.6±1.3</td>
<td>4.0±1.6</td>
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<tr>
<td>FVR (mm Hg/mL per min per 100 mL tissue)</td>
<td>18.2±3.8</td>
<td>24.9±4.1*</td>
<td>28.9±5.8*</td>
</tr>
</tbody>
</table>

All results are presented as mean±SD. *P<0.05 versus normotensive; †P<0.05 versus hypertensive.

Although the vasodilatory effect of ISDN was similar in the normotensive subjects and essential hypertensive patients (Figure 1, bottom). The ratio of maximal ACh-stimulated FBF to maximal ISDN-stimulated FBF was greatest in normotensive subjects and least in patients with pheo (pheo versus essential hypertension versus normotension, 1.1±0.1 versus 1.6±0.2; pheo versus normotension, 1.1±0.1 versus 2.4±0.4; and essential hypertension versus normotension, 1.6±0.2 versus 2.4±0.4; P<0.01 for all). No significant change was observed in the arterial blood pressure or heart rate with the intra-arterial infusion of either ACh or ISDN in any group.

### Study Protocol 2: Effects of Adrenalectomy on Endothelial Function in Patients With Pheo

The baseline clinical characteristics before and after adrenalectomy of the 8 patients with pheo are summarized in the Table. Adrenalectomy significantly decreased serum glucose, plasma, and urinary NE, systolic and diastolic blood pressures, heart rate, and FVR and significantly increased serum insulin.

After adrenalectomy, FBF responses to both ACh and ISDN were enhanced in all patients (maximal FBF: 12.1±2.5 versus 32.2±9.6 mL/min per 100 mL tissue and 11.4±2.1 versus 15.4±3.6 mL/min per 100 mL tissue; P<0.05, respectively; Figure 2). No significant change was observed in the arterial blood pressure or heart rate in response to intra-arterial infusion of either ACh or ISDN before and after adrenalectomy. The ratio of maximal ACh-stimulated FBF to maximal ISDN-stimulated FBF was significantly higher after adrenalectomy than before adrenalectomy (2.1±0.4 versus 1.1±0.1; P<0.05). The increase in maximal FBF response to ACh correlated significantly with the decrease in urinary excretion of NE (r=-0.62, P<0.01; Figure 3) and the decrease in plasma concentrations of NE (r=-0.57, P<0.01). No significant correlation was seen between the increase in maximal FBF response to ACh and changes in blood pressure or variables, such as lipid, glucose, and insulin concentrations, or between these variables and the increase in maximal FBF response to ISDN.
In the present study, we demonstrated that (1) endothelium-dependent vasodilation in the forearm arteries was impaired in patients with essential hypertension compared with normotensive subjects and impaired to a greater extent in patients with adrenal pheo secreting NE; (2) endothelium-independent vasodilation was impaired in patients with pheo compared with normotensive subjects and patients with essential hypertension, and the vasodilatory response in both normotensive subjects and patients with essential hypertension was similar; (3) surgical resection of adrenal pheos improves endothelium-dependent vasodilation in these patients; and (4) there was a significant correlation between the improvement of endothelium-dependent vasodilation and the decrease in NE concentrations.

The forearm vascular response to ACh but not to ISDN was impaired in patients with essential hypertension. These findings are consistent with previous studies that demonstrated that endothelium-dependent vasodilation of brachial, coronary, renal, and small arteries is selectively impaired in patients with essential hypertension compared with normotensive subjects. Patients with adrenal pheo secreting NE are an ideal model for the study of how endothelial function is altered in the presence of the excess vasoconstricting factors. In the present study, basal NO levels were similar in patients with pheo and the other groups. In contrast, stimulation with ACh blunted the endothelium-dependent vasodilation probably through a decrease in NO release in pheo patients. In addition, ISDN-induced vasodilation via smooth muscle cells was attenuated in these patients, although to a lesser degree than the endothelium-dependent vasodilatory response. Both ACh- and ISDN-stimulated vasodilation were blunted in patients with adrenal pheo secreting NE compared with patients with essential hypertension and normotensive subjects, indicating that both endothelial and smooth muscle function is impaired and may be restored after surgery in patients with pheo.

Several investigators have shown the existence of an interaction between NO and NE in intact endothelium. Greenberg et al reported that cyclic guanosine monophosphate (cGMP) may be a prejunctional regulator of NE as a sympathetic neurotransmitter in the vasculature. These findings suggest that both NO and cGMP may act as endogenous inhibitors of NE release in vascular smooth muscle. However, this compensatory mechanism of NE release may not naturally function under excess levels of NE. In the present study, the improvement of endothelium-dependent vasodilation significantly correlated with the decrease in NE concentrations. One possible mechanism by which surgical resection of NE-secreting tumors augments endothelium-dependent vasodilation is a decrease in NE release. An imbalance between NO and NE may directly result in conditions associated with the endothelial dysfunction in humans.

It is widely known that diseases of abnormal glucose metabolism, such as diabetes mellitus, are associated with endothelial dysfunction. In pheo patients, increases in serum glucose and decreases in insulin were caused by excess NE. Although serum glucose and insulin concentrations returned to levels similar to normotensive subjects after surgical resection of the adrenal tumor, there was no significant correlation between the improvement in endothelial function and changes in serum glucose and insulin. In addition, abnormal lipid metabolism, such as what occurs with hypercholesterolemia, is associated with endothelial dysfunction. In the present study, the concentrations of total and LDL cholesterol were higher, although not significantly, in patients with pheo compared with the other groups. There was no correlation between the improvement in endothelial function and changes in the lipid profile, suggesting that modification of the lipid profile does not contribute to the adrenalectomy-induced restoration of endothelial function.

Endothelial function becomes impaired as blood pressure increases, and the degree of dysfunction is related to the severity of the hypertension. It is expected that endothe-
lial dysfunction is improved by antihypertensive therapy. However, several experimental and clinical studies have found conflicting results concerning the relationship between the reduction in blood pressure and the improvement in endothelial function. Although in the present study, adrenalectomy acutely decreased blood pressure in patients with pheo, changes in blood pressure did not correlate with the improvement of FBF response to either ACh or ISDN. In previous studies, we and other investigators showed that although clinically effective antihypertensive therapy, such as angiotensin converting enzyme inhibitors and aerobic exercise, have restored resistance artery endothelial function of forearm circulation in patients with essential hypertension, there is no significant correlation between the degree of reduction in blood pressure and the augmentation of endothelium-dependent vasodilation. Therefore, a reduction in blood pressure per se may not be involved in the restoration of resistance artery endothelial function in the forearm circulation.

Although relatively rare, impairment of smooth muscle response to NO may occur in patients with pheo in contrast to previous observations that such endothelium-independent vasodilation is not impaired in atherosclerosis, coronary heart disease, hypertension (including essential hypertension, primary aldosteronism, and renovascular hypertension), and even heart failure. Instead of vascular smooth muscle function, endothelium-dependent vasodilation has been shown to be impaired in these other conditions. This may be explained by the fact that circulating NE mainly stimulates α-adrenergic receptors, located on vascular smooth muscle, causing vasoconstriction and thereby elevating blood pressure in patients with pheo. The excess circulating NE may contribute, at least in part, to the blunted smooth muscle response to exogenous NO. However, because there was no significant correlation between the improvement of ISDN-stimulated vasodilation and the decrease in NE concentrations after adrenalectomy, it is unlikely that the removal of excess NE directly restores endothelium-independent vasodilation after adrenalectomy.

Conversely, our results suggest that adrenalectomy-induced structural alterations may occur in patients with pheo. Interestingly, ISDN-stimulated vasodilation was quickly restored after adrenalectomy. We confirmed this restoration within 2 weeks after adrenalectomy in 5 of the 8 patients with laparoscopic adrenalectomy. Although we cannot deny the possibility that vascular structural adjustments after adrenalectomy contribute to the normalization of vascular response to ISDN, it is unlikely that structural changes, including vascular smooth muscle cell proliferation and remodeling, occur in such a short period.

Study Limitations

In the present study, the number of patients with pheo was relatively small. Nonetheless, we observed a marked augmentation of endothelium-dependent vasodilation after adrenalectomy in patients with pheo.

The use of α (β)-adrenergic receptor blockers would allow more specific conclusions concerning the effects of NE on endothelium-dependent and -independent vasodilation in the forearm circulation.

It is widely known that various vasoconstricting factors other than NE affect endothelium-dependent and -independent vasodilation in humans. We confirmed that circulating levels of endothelin-1 and angiotensin II were normal and were not changed after adrenalectomy (1.9±0.2 to 1.8±0.3 pg/mL and 14.2±6.9 to 15.3±7.1 pg/mL) in patients with pheo. However, we cannot deny the possibility that other vasoconstrictors contribute to impaired ACh- and ISDN-stimulated vasodilation and were restored after adrenalectomy in patients with pheo.

Conclusions

Increased secretion of NE impairs both endothelium-dependent and -independent vasodilation in forearm circulation, although excess NE has a predominate effect on endothelium-dependent vasodilatory mechanisms in humans. The resection of an NE-secreting tumor restores both endothelium-dependent and -independent vasodilation in patients with pheo.

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References


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