Contribution of Endogenous Nitric Oxide to Basal Vasomotor Tone of Peripheral Vessels and Plasma B-Type Natriuretic Peptide Levels in Patients With Congestive Heart Failure

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OBJECTIVES

We examined whether a relationship exists between the vasoconstrictive response to endogenous nitric oxide (NO) synthesis inhibition and the severity of heart failure in patients with congestive heart failure (CHF).

BACKGROUND

Controversy exists as to whether the vasoconstrictive response to NO synthesis inhibition in patients with CHF is comparable to that in normal subjects or is enhanced.

METHODS

Forearm blood flow (FBF) and calculated forearm vascular conductance (FVC) were obtained using plethysmography before and after administration of the NO synthesis inhibitor L-NMMA (N\textsuperscript{G}-monomethyl-L-arginine) in 40 patients with CHF due to dilated cardiomyopathy and in 16 normal control subjects. Basal plasma B-type natriuretic peptide (BNP) and nitric oxide concentrations were measured in all subjects.

RESULTS

Plasma BNP and nitrite/nitrate (NOx) levels in the patients group were significantly greater and baseline FBF was significantly less. Administration of L-NMMA significantly decreased FBF and FVC in both groups. The percent changes in FBF (%FBF) and FVC (%FVC) from the baseline after L-NMMA correlated significantly with plasma BNP level (%FBF: r = 0.72; %FVC: r = 0.76; both p < 0.001). Percent changes in both FBF and FVC were greater in patients with BNP $\geq$ 100 pg/ml than in normal subjects; however, in patients with BNP < 100 pg/ml they were comparable to those in normal subjects.

CONCLUSIONS

Vasoconstrictive response to L-NMMA in patients with CHF was preserved or enhanced in proportion to the basal plasma BNP level, indicating a close relationship between the contribution of endogenous NO to basal vasomotor tone and the severity of heart failure.

(J Am Coll Cardiol 2000;36:1605–11) © 2000 by the American College of Cardiology

Endothelium-derived nitric oxide (NO) plays a pivotal role in the regulation of the vasomotor tone of peripheral vessels. Vallance et al. (1) first showed that basal release of NO, which accounts for the biological activity of endothelium-derived relaxing factor, contributes to the control of regional blood flow in humans, using N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA), a selective inhibitor of NO synthesis from L-arginine (2). In patients with congestive heart failure (CHF), agonist-induced NO-mediated vasodilatation in response to muscarinic stimulation is impaired in the peripheral circulation (3–5). By contrast, there are conflicting results showing that vasoconstrictive response to L-NMMA increases (6), is normal (7), or increases (8–10) in patients with CHF compared with that of normal subjects. Differences in the results of these previous studies may be related to differences in the severity or the etiology of heart failure in the study populations. A recent study demonstrated that there was no difference in vasoconstrictive response to L-NMMA in patients with moderate heart failure in New York Heart Association (NYHA) functional class I and class II and normal subjects but that the vasoconstrictive response in patients with severe heart failure in NYHA functional class III and class IV was significantly less than that in normal subjects (11).

In the present study, to examine whether the basal production of NO in patients with CHF might be related to the severity of heart failure, we used the basal plasma level of B-type natriuretic peptide (BNP) for classifying the degree of heart failure instead of using NYHA classification, because the plasma BNP level is more objective than NYHA classification. Although both atrial natriuretic peptide (ANP) and BNP levels increase in patients with CHF, the circulating level of BNP has been reported to increase in proportion to the functional severity of heart failure to a greater extent than that of ANP (12,13). We therefore designed this study to determine whether the degree to which L-NMMA affects peripheral vasomotor tone is related to the plasma BNP level of patients with CHF.
Methods

Subjects. Forty-six patients with CHF due to idiopathic dilated cardiomyopathy who had been admitted to our hospital and 16 age- and gender-matched healthy control volunteers were enrolled in this study. The patient group included 28 men and 18 women with a mean age of 60 years (range 44 to 76 years). The control group consisted of 10 men and 6 women with a mean age of 58 years (range 36 to 78 years) who showed no abnormalities upon physical examination, electrocardiography, chest radiography, and routine blood tests including fasting blood sugar and serum cholesterol. Current smokers and subjects who had smoked within two years were excluded from this study.

Patients with renal dysfunction (serum creatinine ≥1.2 mg/dl) were not enrolled in the study, because the plasma level of BNP is affected by renal function (14). All patients underwent cardiac catheterization to measure cardiac hemodynamics and to perform left ventriculography and coronary angiography. Two patients with organic coronary stenotic lesions >75% were excluded from the study. Four patients with transient atrial fibrillation detected within seven days before the study were also excluded because it has been proposed that atrial fibrillation decreases endothelium-dependent vasodilatation and NO production (15). Mean left ventricular ejection fraction obtained by catheterization in the remaining 40 patients with CHF was 26 ± 8%. Cardiac index was 2.16 ± 0.71 liter/min/m², and mean pulmonary capillary wedge pressure was 19 ± 8 mm Hg. In terms of the clinical severity of heart failure, the patients were in NYHA functional classes I to III. All patients had been clinically stable for at least two weeks before the study, with no signs of pulmonary congestion or peripheral edema. Background therapies were digoxin (n = 29), diuretics (n = 30), angiotensin-converting enzyme (ACE) inhibitors (n = 20), nitrates (n = 12), calcium antagonists (n = 5) and anticoagulants (n = 13). All vasodilating medications and anticoagulants were discontinued two or three days before the day of the study, and digitalis and diuretics were stopped on the day of the study. During this period, the patients were closely monitored for any sign of aggravation of heart failure. All patients and control subjects gave their written, informed consent to participation in the study, which had been approved by the Human Subjects Research Committee of Shimane Medical University Hospital.

Procedures and protocols. All participants were instructed to abstain from eating food and drinking caffeinated beverages for at least 12 h before the study, which was performed in the supine position in an air-conditioned room at a temperature of 25–26°C. Forearm blood flow (FBF) was determined by venous occlusion plethysmography as described elsewhere (16). Briefly, under local anesthesia with 1% lidocaine, the brachial artery of the nondominant arm (the left arm) was cannulated with a 22-gauge polyethylene catheter (RA-4122, Arrow, Reading, Pennsylvania) for pressure monitoring and drug infusion. A 24-gauge polyethylene catheter was placed in the basilica vein for venous blood sampling. This arm was slightly elevated above the level of the right atrium, and a mercury-filled silicone strain-gauge was placed in the widest part of the forearm. The strain-gauge was connected to a Hokanson EC-5R Plethysmograph (Hokanson, Bellevue, Washington) that was calibrated to measure the percent change in volume and connected in turn to a chart recorder to record the flow measurements. For each measurement, a cuff placed around the upper arm was inflated to 40 mm Hg with a rapid cuff inflator (E-10, Hokanson) to occlude venous outflow from the extremity. A wrist cuff was inflated to suprasystolic pressure 1 min before each measurement to exclude the hand circulation. Flow measurements were recorded for 5 s every 15 s, and four readings were obtained for each mean value. Systemic blood pressure was measured with a cuff sphygmomanometer placed on the contralateral arm, and arterial pressure on the side of FBF measurements was continuously monitored with a Life Scope 12 polygraph (Nihonkoden, Tokyo, Japan). Forearm vascular conductance (FVC) was calculated by dividing FBF by the mean arterial pressure.

After a 20-min rest period, blood samples were obtained for measurements of baseline ANP, BNP and nitric oxide (NOx: nitrite/nitrate) levels. After baseline FBF was measured, L-NMMA (100 μmol, Clinalfa, Laüelfingen, Switzerland) was infused over 5 min. Five minutes after completion of the infusion, FBF was measured again.

Measurement of plasma ANP, BNP, and NOx concentrations. Venous plasma ANP and BNP levels were determined using commercially available radioimmunoassay kits (Shionoria, Shionogi, Osaka, Japan). In this assay, the minimal detectable quantities of ANP and BNP are 5 pg/ml and 2 pg/ml, respectively, and cross-reactivities with other natriuretic peptides are <0.001% on a molar basis. Venous nitrate/nitrate (NOx) levels were measured using the fluorometric method reported previously (17,18). Briefly, plasma was incubated with NADPH and Aspergillus niger nitrate reductase (Sigma Chemical, St. Louis, Missouri) and subsequently with 2,3-diaminonaphthalene (Dojindo Labs, Kumamoto, Japan). Fluorescence intensity was measured with a Hitachi 850 fluorescence spectrophotometer (Hitachi Co., Tokyo, Japan). Nitrate standards (>98% pure, Sigma Lake, St. Louis, Missouri) were used for calibration.

Antagonists (n = 5) and angiotensin-converting enzyme (ACE) inhibitors (n = 20), nitrates (n = 12), calcium antagonists (n = 5) and anticoagulants (n = 13). All vasodilating medications and anticoagulants were discontinued two or three days before the day of the study, and digitalis and diuretics were stopped on the day of the study. During this period, the patients were closely monitored for any sign of aggravation of heart failure. All patients and control subjects gave their written, informed consent to participation in the study, which had been approved by the University Hospital. Methods and protocols. All participants were instructed to abstain from eating food and drinking caffeinated beverages for at least 12 h before the study, which was performed in the supine position in an air-conditioned room at a temperature of 25–26°C. Forearm blood flow (FBF) was determined by venous occlusion plethysmography as described elsewhere (16). Briefly, under local anesthesia with 1% lidocaine, the brachial artery of the nondominant arm (the left arm) was cannulated with a 22-gauge polyethylene catheter (RA-4122, Arrow, Reading, Pennsylvania) for pressure monitoring and drug infusion. A 24-gauge polyethylene catheter was placed in the basilica vein for venous blood sampling. This arm was slightly elevated above the level of the right atrium, and a mercury-filled silicone strain-gauge was placed in the widest part of the forearm. The strain-gauge was connected to a Hokanson EC-5R Plethysmograph (Hokanson, Bellevue, Washington) that was calibrated to measure the percent change in volume and connected in turn to a chart recorder to record the flow measurements. For each measurement, a cuff placed around the upper arm was inflated to 40 mm Hg with a rapid cuff inflator (E-10, Hokanson) to occlude venous outflow from the extremity. A wrist cuff was inflated to suprasystolic pressure 1 min before each measurement to exclude the hand circulation. Flow measurements were recorded for 5 s every 15 s, and four readings were obtained for each mean value. Systemic blood pressure was measured with a cuff sphygmomanometer placed on the contralateral arm, and arterial pressure on the side of FBF measurements was continuously monitored with a Life Scope 12 polygraph (Nihonkoden, Tokyo, Japan). Forearm vascular conductance (FVC) was calculated by dividing FBF by the mean arterial pressure.

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Abbreviations and Acronyms

ANP = atrial natriuretic peptide
ANOVA = analysis of variance
BNP = B-type natriuretic peptide
CHF = congestive heart failure
FBF = forearm blood flow
FVC = forearm vascular conductance
L-NMMA = Nω-monomethyl-L-arginine
NO = nitric oxide
NOx = nitrate/nitrate
NYHA = New York Heart Association
Increased NO Production in Patients With Severe Heart Failure  

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**Table 1. Clinical Characteristics of Study Population**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>10/6</td>
<td>23/7</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>58 ± 11</td>
<td>59 ± 10</td>
</tr>
<tr>
<td>NYHA functional class I (n)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>NYHA functional class II (n)</td>
<td>(14)</td>
<td></td>
</tr>
<tr>
<td>NYHA functional class III (n)</td>
<td>(14)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>177 ± 25</td>
<td>180 ± 29</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>78 ± 34</td>
<td>90 ± 32</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>53 ± 8</td>
<td>56 ± 7</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>109 ± 24</td>
<td>110 ± 27</td>
</tr>
<tr>
<td>Fasting blood sugar (mg/dl)</td>
<td>91 ± 7</td>
<td>96 ± 10</td>
</tr>
<tr>
<td>Atrial natriuretic peptide (pg/ml)</td>
<td>7 ± 3</td>
<td>74 ± 81*</td>
</tr>
<tr>
<td>Brain natriuretic peptide (pg/ml)</td>
<td>4 ± 2</td>
<td>244 ± 187*</td>
</tr>
<tr>
<td>Nitrite and nitrate (µmol/liter)</td>
<td>4 ± 3</td>
<td>17 ± 15*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>57 ± 7</td>
<td>64 ± 11</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>115 ± 9</td>
<td>118 ± 10</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>67 ± 7</td>
<td>69 ± 14</td>
</tr>
</tbody>
</table>

Values are mean ± SD. CHF = congestive heart failure. *p < 0.001 vs. corresponding value in control.

**Statistical analysis.** Data are expressed as mean ± SD unless otherwise indicated. Intergroup differences were analyzed with the chi-square test or unpaired t tests for baseline characteristics, except for ANP, BNP and NOx levels. Either the Mann-Whitney U test or Kruskall-Wallis analysis of variance (ANOVA) followed by Scheffe’s post hoc test was used to compare the nonparametric variables ANP, BNP and NOx. Responses to l-NMMA were compared with two-way (group and drug treatment) ANOVA for repeated measures. When significant differences were observed, comparison within groups or drug treatments was performed with one-way ANOVA followed by the Scheffe test. Correlations between measured variables of interest were determined by simple linear regression analysis, and differences in changes in FBF and FVC after administration of l-NMMA were compared using analysis of covariance with baseline FBF or FVC as a covariate to adjust for differences in basal FBF and FVC. A value of p < 0.05 was considered statistically significant.

**RESULTS**

Clinical characteristics of the study population. Baseline clinical characteristics of the study participants are shown in Table 1. Blood pressure and heart rate tended to be higher in the CHF patients than in the control subjects, but the differences were not significant. The CHF patients showed evidence of hormonal activation, in the form of distinctly higher plasma ANP and BNP levels, than did control subjects (p < 0.001). Plasma NOx levels were also significantly higher in the CHF patients than in controls (p < 0.001).

Effects of l-NMMA on baseline FBF and FVC. Individual l-NMMA–induced changes in FBF are shown in Figure 1, and mean values by BNP level are listed in Table 2. Baseline values for FBF and FVC were 3.66 ± 0.72 ml/min/dl and 4.1 ± 0.8 × 10⁻² ml/min/dl/mm Hg in the control group and 2.74 ± 0.76 ml/min/dl and 3.1 ± 1.3 × 10⁻² ml/min/dl/mm Hg in the CHF patient group (all patients). Differences between the two groups were significant (FBF: p = 0.002; FVC: p < 0.001). Administration of l-NMMA did not alter the heart rate or the blood pressure in either group, but it significantly lowered baseline FBF to 2.54 ± 0.55 ml/min/dl (p < 0.001) in the control group and to 1.89 ± 0.63 ml/min/dl (p < 0.001) in the CHF group. Also, FVC significantly decreased after l-NMMA in both groups (p < 0.001). Average decrease in both FBF and FVC did not differ between the control and patient groups (absolute change in FBF: −1.12 ± 0.28 vs. −0.87 ± 0.39 ml/min/dl, p = 0.08; percent change in FBF: −31 ± 5% vs. −32 ± 11%, p = 0.38; percent change in FVC: −34 ± 5% vs. −38 ± 13%, p = 0.33).

We then compared five patient groups, based on their plasma BNP levels: BNP <50 pg/ml; 50 ≤ BNP <100 pg/ml; 100 ≤ BNP <200 pg/ml; 200 ≤ BNP <300 pg/ml; and BNP ≥ 300 pg/ml. The l-NMMA significantly lowered the FBF and FVC in patients in the BNP ≤ 100 pg/ml range. By contrast, in patients with BNP <100 pg/ml, FBF and FVC tended to decrease after administration of l-NMMA, but the differences were not significant. Percent changes in FBF and FVC in patients with BNP <100 pg/ml were comparable to those in the control group, whereas they were significantly greater in patients with BNP ≥ 100 pg/ml.

In both groups, FBF measured on the contralateral forearm did not change significantly after administration of l-NMMA.

Relationship between percent changes in FBF and FVC after l-NMMA and plasma BNP levels. The percent changes in FBF after l-NMMA closely correlated with the plasma BNP levels (n = 56; r = 0.72; p < 0.001), and the percent changes in FVC also correlated significantly with...
the plasma BNP levels (n = 56; r = 0.76; p < 0.001) (Fig. 2).

**Relationship between percent changes in FBF and FVC after L-NMMA and plasma NOx levels.** The percent changes in FBF and FVC after L-NMMA infusion correlated weakly but significantly with the levels of plasma NOx levels (n = 56; r = 0.60, p = 0.003 and r = 0.66, p < 0.001, respectively; both p < 0.001) (Fig. 3). By contrast, the absolute changes in FBF after L-NMMA infusion did not correlate with either NOx or BNP levels (n = 56; r = 0.08, p = 0.96; and r = 0.01, p = 0.93, respectively), whereas it did correlate significantly with baseline FBF (n = 56; r = 0.65; p < 0.001) (not shown).

**DISCUSSION**

The present data demonstrate that the contribution of endogenous NO to the basal vasomotor tone of the peripheral vessels in the CHF patients varied according to the plasma BNP level. Thus, the percent changes in FBF and FVC after L-NMMA infusion in patients with CHF with BNP <100 pg/ml were comparable to those in control subjects, and in patients with BNP ≥100, they were significantly greater. These findings suggest that the basal production and release of NO in patients with CHF may vary according to the severity of heart failure. Our results may help to resolve certain issues in previous studies that investigated different patient populations.

**Previous studies in humans.** Drexler et al. (8) reported that an L-NMMA–induced decrease in FBF calculated from vessel diameter and blood flow velocity using echocardiography was greater in patients with CHF (46%) than in normal control subjects (38%), and they emphasized that blocking of basal NO release caused an exaggerated vasoconstrictive response in patients with CHF. Similarly, following systemic administration of L-NMMA, Habib et al. (9) found the highest increase in systemic vascular resistance after L-NMMA administration in those CHF patients with the highest basal systemic vascular resistance. In addition, Winlaw et al. (10) reported measurements of increased plasma nitrate levels in patients with CHF. These previous observations suggest that the production and release of NO in the basal state is enhanced in patients with CHF.

By contrast, Kubo et al. (7) reported no significant differences between normal subjects and patients with CHF in either absolute or percent change in FBF during infusion of graded doses of L-NMMA. In that study, the mean

**Table 2. Effects of L-NMMA on FBF and FVC in Range of Plasma BNP Level**

<table>
<thead>
<tr>
<th></th>
<th>BNP (pg/ml)</th>
<th>NOx (µmol/liter)</th>
<th>FBF (ml/min/dl)</th>
<th>FVC (×10⁻² ml/min/dl/mm Hg)</th>
<th>% Change</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Baseline</td>
<td>L-NMMA</td>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
<td>4 ± 2</td>
<td>4 ± 3</td>
<td>3.66 ± 0.72</td>
<td>2.54 ± 0.55‡</td>
<td>−31 ± 5</td>
</tr>
<tr>
<td>CHF all patients</td>
<td>40</td>
<td>244 ± 187**</td>
<td>17 ± 15*</td>
<td>2.74 ± 0.76*</td>
<td>1.89 ± 0.63‡</td>
<td>−32 ± 11</td>
</tr>
<tr>
<td>BNP &lt; 50</td>
<td>7</td>
<td>31 ± 13</td>
<td>5 ± 4</td>
<td>2.96 ± 0.87</td>
<td>2.31 ± 0.66</td>
<td>−22 ± 12</td>
</tr>
<tr>
<td>50 ≤ BNP &lt;100</td>
<td>7</td>
<td>77 ± 20*</td>
<td>14 ± 15</td>
<td>2.95 ± 0.54</td>
<td>2.37 ± 0.33</td>
<td>−21 ± 14</td>
</tr>
<tr>
<td>100 ≤ BNP &lt;200</td>
<td>10</td>
<td>157 ± 30*</td>
<td>18 ± 11*</td>
<td>2.88 ± 0.66</td>
<td>1.87 ± 0.50†</td>
<td>−37 ± 7</td>
</tr>
<tr>
<td>200 ≤ BNP &lt;300</td>
<td>7</td>
<td>248 ± 19*</td>
<td>19 ± 10*</td>
<td>2.85 ± 0.78</td>
<td>1.78 ± 0.46†</td>
<td>−43 ± 8</td>
</tr>
<tr>
<td>300 ≤ BNP</td>
<td>9</td>
<td>462 ± 189**</td>
<td>30 ± 20*</td>
<td>2.13 ± 0.82**</td>
<td>1.18 ± 0.46†</td>
<td>−47 ± 9</td>
</tr>
</tbody>
</table>

Values are mean ± SD. CHF = congestive heart failure; FBF = forearm blood flow; FVC = forearm vascular conductance; % change = percent change in FBF or FVC from baseline after L-NMMA.

* p < 0.01 vs. corresponding value in control. ** p < 0.001 vs. corresponding value in control. † p < 0.01 vs. corresponding baseline value. ‡ p < 0.001 vs. corresponding baseline value.
values for percent change in FBF at the highest dose of L-NMMA were 33% in 18 normal subjects and 35% in 10 patients with NYHA functional class II to IV heart failure using plethysmography. Their results are in agreement with our results.

Recently, Carville et al. (11) demonstrated that vasoconstrictive response to L-NMMA varied according to the NYHA classification of heart failure. Thus, the percent change in FBF after L-NMMA administration in patients with moderate heart failure (NYHA functional class I or II) was similar (57%) to that in control subjects (55%), and that in patients with severe heart failure (NYHA functional class III or IV) was reduced (43%). Those results, however, are not entirely consistent with those of previous studies and the present study. The difference in results suggests that NYHA classification of heart failure may be inadequate because it is subjective. The use of plasma BNP levels in the present study provides a more objective measure for the classification of the severity of heart failure.

These previous studies and the present study show that vasoconstrictive response to L-NMMA in patients with CHF is preserved or exaggerated, suggesting that basal NO production and release in patients with CHF may be normal or increased. However, Katz et al. (6) demonstrated that administration of L-NMMA significantly lowered FBF by 25% in normal subjects but did not have this effect in patients with CHF. Those results accord with the majority of previous experimental studies (19,20). We cannot explain the differences between the findings of the Katz et al. study and the present data by differences in the severity of heart failure, because basal FBF in the patients in the Katz et al. study was low (2.3 ± 0.3 ml/min/dl), suggesting moderate or severe heart failure. One possible reason might be that a smaller dose of L-NMMA (20 μmol) was used than in the present study (100 μmol) and previous studies (60 to 145 μmol) (7–9,11). Kubo et al. (7) showed that decreases in FBF during infusion of low doses of L-NMMA were significant but smaller than during infusion of high doses of L-NMMA, and those findings might support this explanation.

Enhanced production of NO in patients with severe heart failure. Nitric oxide is synthesized from L-arginine by calcium-dependent and -independent NO synthases, a family of isoenzymes with distinct functional, biochemical, and regulatory properties (21,22). The calcium-dependent NO synthase in the endothelial cells plays an important role in the regulation of peripheral circulation. However, recent studies have shown the development of endothelial dysfunction in the peripheral resistance vessels of patients with CHF (3–5,8) and a decrease in endothelial-NO synthase expression in a dog model of heart failure (23), suggesting that endothelial NO production is impaired in CHF. In this respect, it seems probable that the increased basal release of NO originates from another isoform of NO synthase, an inducible calcium-independent NO synthase (24), which can produce large amounts of NO. Indeed, in the present study, the plasma NO_{x} level in the patient with the highest level of NO_{x} (64 μmol/liter) was approximately 20 times higher than that in normal subjects. This difference may not be entirely accounted for by increased endothelial NO production.

In preliminary studies, we measured FBF and venous NO_{x} levels before and during intra-arterial infusion of 30 μg/min of acetylcholine in four normal subjects (data not shown). Although we observed a greater than 10-fold increase in blood flow over the baseline in response to acetylcholine, the increase in NO_{x} was limited to approximately 80%. This finding suggests that the large amount of NO in patients with severe heart failure might be produced not by endothelial NO synthase but by inducible NO synthase. However, our results do not allow us to draw any conclusions as to whether the increased NO in CHF patients is produced in the forearm tissue and/or in the myocardium.

A recent study demonstrated that gene expression of inducible NO synthase was increased in the skeletal muscle of patients with severe heart failure (25), suggesting the possibility of enhanced local production of NO in the forearm tissue. In the present study, the enhanced vasoconstrictive response to L-NMMA proportional to the severity of heart failure is consistent with the hypothesis of an association between severe heart failure and the expression of inducible NO synthase in the peripheral vascular wall (8).

In the present study, by contrast, we found a nonsignificant trend toward a lesser effect of L-NMMA on FBF and FVC in patients with mild heart failure (BNP <100 pg/ml) compared with control subjects. It is difficult to assess the meaning of this finding from our data. However, it might possibly be suggested that in patients with mild heart failure, NO produced by inducible NO synthase is not present, whereas endothelium-derived NO production is still impaired, resulting in a decrease in NO production.

We believe that basal production and release of NO is enhanced in severe heart failure. It should be noted, however, that heightened vasoconstrictor tone is present in heart failure, which, in this setting, may accentuate the effects of any additional vasoconstricting agent. Because we did not measure the diameter of brachial artery, the possibility of a direct constricting effect of L-NMMA on the conduit vessel cannot be excluded. Previous investigators reported that L-NMMA had no direct constrictive effect on vascular smooth muscle (26,27). However, further studies of basal NO production in heart failure are needed, including comparisons of the constrictive effects of a control agent and of L-NMMA, to strengthen these conclusions.

Clinical implications. The increased basal production and release of NO demonstrated in the present study may play an important role in reducing the vascular resistance of the peripheral vessels and maintaining adequate tissue perfusion. However, these large amounts of NO may have adverse consequences. The force of contraction in the skeletal muscles is modulated by NO through impaired
Ca$^{2+}$ activation of actin filaments, resulting in decreased myofibrillar calcium sensitivity. Therefore, in patients with severe heart failure, the increased availability of NO within the skeletal muscle may affect the contractile force, resulting in decreased capacity for exercise. Recent studies in humans have shown that impaired endothelial function in the peripheral vessels may be corrected by a variety of interventions (28–33), though the clinical benefits of such improvements in endothelial function have not been conclusively demonstrated in patients. Therapeutic trials focused on the increased production of NO in patients with severe heart failure should also be planned.

**Study limitations.** In the present study, we measured plasma NO$_x$ levels to assess basal NO production. However, plasma NO$_x$ concentration is determined primarily from exogenous sources such as diet (34), therapeutic medical regimens (10) and renal excretion (35). Although all vasodilating drugs were discontinued two or three days before the date of the study, we cannot exclude the possibility that exogenous nitrate and/or nitrite are included in the measurements. Another limitation is the question of whether the percent changes in FBF and FVC after 1–NMMA precisely reflect basal NO production and release. However, the percent changes in FBF and FVC after 1–NMMA correlated weakly but significantly with plasma NO$_x$ levels, while the absolute changes in FBF did not. These findings suggest that the percent changes in FBF and FVC after 1–NMMA partly reflect basal NO production. Still, NO$_x$ measurements before and after administration of 1–NMMA, which were performed in a previous study (7), will be needed to clarify local NO production in patients with heart failure.

**Conclusions.** The present study demonstrated that vasconstrictive response to 1–NMMA in patients with CHF was preserved or enhanced in proportion to the plasma BNP level, indicating a close relationship between the contribution of NO to basal vasomotor tone and the severity of heart failure. However, the mechanism behind these observations remains to be clarified, and further studies are needed to confirm these findings.

**REFERENCES**

27. Rees DD, Palmer RM, Moncada S. Role of endothelium-derived...