Endothelium-Dependent Vasodilation Is Attenuated in Patients With Heart Failure

Spencer H. Kubo, MD; Thomas S. Rector, PhD; Alan J. Bank, MD; Randall E. Williams, MD; and Steven M. Heifetz, MD

Background. Endothelial cells produce a number of substances, collectively termed endothelium-derived relaxing factor (EDRF), that promote local relaxation of vascular smooth muscle. Although studies have demonstrated defects in endothelium-dependent vasodilation in animal models of hypertension, atherosclerosis, and heart failure, there are only limited data from human subjects because of the difficulty in obtaining fresh vascular segments.

Methods and Results. To address the hypothesis that endothelium-dependent vasodilation is attenuated in patients with heart failure, we measured forearm blood flow responses to the intra-arterial administration of methacholine, a known stimulus of EDRF release through muscarinic receptors. In 14 normal subjects, a dosage range of methacholine increased forearm blood flow by 5.26±0.63, 10.50±0.63, and 13.22±0.86 ml/min/100 ml forearm volume (FAV); these responses were 1.98±0.46, 5.48±0.79, and 8.50±1.53 ml/min/100 ml FAV in 14 patients with heart failure. When pooled over all doses, the responses were strikingly less in the patients with heart failure (5.32±0.31 versus 9.52±0.60 ml/min/100 ml FAV; p=0.0003). In a second study, the average difference in forearm blood flow responses between patients with heart failure and normal subjects with methacholine was significantly greater than the average difference between the groups with nitroprusside (4.04±1.10 versus 2.20±0.71 ml/min/100 ml FAV; p=0.04). The decreased methacholine responses in the patients with heart failure were not related to age (r=0.39; p=NS) or etiology because there was no difference in the responses between patients with ischemic heart disease and those with idiopathic cardiomyopathy.

Conclusions. These data suggest that endothelium-dependent vasodilation is attenuated in patients with heart failure. Although the mechanisms of the decreased endothelium-dependent responses in heart failure are not known, this impaired local vasodilation may contribute to abnormalities in vasoconstriction that are characteristic of heart failure. (Circulation 1991;84:1589-1596)

One of the common hemodynamic findings in patients with heart failure is heightened vasoconstriction, which is related to activation of several circulating neurohormonal systems. However, because there are very poor correlations between plasma levels of these neurohormones and systemic vascular resistance, there are probably other systems that modulate vascular tone. It is now well known that endothelial cells produce a number of substances, collectively termed endothelium-derived relaxing factor (EDRF), that promote local relaxation of underlying vascular smooth muscle in the basal state as well as in response to stimuli such as acetylcholine. More recent studies using isolated vascular segments have demonstrated defects in endothelium-dependent vasodilation in animal models of hypertension, atherosclerosis, and heart failure. Endothelium-dependent vasodilation has also been demonstrated in humans, but data from patients with these clinical conditions are limited, in large part because of the difficulty in obtaining fresh vascular segments. Because of well-known species differences, clinical studies are critically important in determining the pathophysiological significance of this local vasodilating mechanism. One approach used to study vascular reactivity in patients measures vaso relaxant responses in terms of changes in flow or vessel diameter after the administration of subsystemic doses of pharmacological probes that are known to stimulate EDRF. There are...
now data demonstrating defects in endothelium-dependent vasodilation in patients with atherosclerosis and hypertension.21-25 However, to our knowledge, there are few reports assessing endothelium-dependent vasodilation in patients with heart failure. We therefore used the isolated forearm model to measure forearm blood flow responses to the intraarterial administration of methacholine, a known stimulus of EDRF release through muscarinic receptors. We also compared these responses with the effects of nitroprusside, an endothelium-independent nitrovasodilator, in both patients with heart failure and normal subjects to address the hypothesis that endothelium-dependent vasodilation is attenuated in patients with heart failure.

Methods

Study Population

The present study consisted of two separate protocols, each of which included patients with heart failure and normal subjects (Table 1). The first protocol compared the forearm blood flow response to methacholine in 14 patients with heart failure with that in 14 normal subjects. To assess the degree of specificity of impaired endothelium-dependent vasodilation, a second protocol consisting of 10 patients with heart failure and eight normal subjects was conducted. These groups received methacholine and nitroprusside, an endothelium-independent nitrovasodilator.

A total of 24 patients with heart failure were studied (23 men and one woman). All patients had left ventricular systolic dysfunction, with ejection fractions as measured by nuclear heart scan ranging between 9% and 40% (mean±SD, 19±8%). The etiology of heart failure was related to ischemic heart disease in 14 patients as defined by a history of myocardial infarction, coronary artery bypass graft surgery, or coronary angiography demonstrating more than 70% obstruction in one or more coronary arteries. Ten patients were diagnosed as having an idiopathic cardiomyopathy in the absence of identifiable causes of ventricular dysfunction. All patients were clinically stable without acute decompensations and had symptoms of New York Heart Association functional class II (n=3) or III (n=21) heart failure. Patients were either admitted to the Clinical Research Center for monitoring 1 day before the study or hospitalized for evaluation as potential cardiac transplant recipients. All patients were treated with digoxin and diuretics, which were withheld on the morning of the study. Vasodilating therapy with a converting enzyme inhibitor (n=19) or nitrates (n=5) was withdrawn 24-48 hours before the study.

A total of 22 normal male subjects were recruited for the control group. All subjects were free of medical illnesses, heart disease, and hypertension as determined by medical history, physical examination, routine blood tests, and electrocardiogram. All patients and subjects signed informed consent, and this study was approved by the Committee on the Use of Human Subjects in Research.

Isolated Forearm Model

All studies were performed in a temperature-controlled room. Each patient ate a light breakfast on the morning of the study. The isolated forearm model26,27 consists of an 18-gauge cannula placed into the brachial artery of the nondominant arm for pressure recording and infusion of the study drugs using a Harvard infusion pump. Forearm blood flow was measured using a mercury-in-Silastic strain-gauge placed around the upper third of the forearm and connected to an electronically calibrated plethysmograph (Hokanson ECS). Five consecutive flow measurements were averaged and expressed in milliliters per minute per 100 ml of forearm volume (FAV). Heart rate was continuously recorded using a precordial electrocardiographic lead. Systolic and diastolic blood pressures were also recorded in the contralateral arm with a cuff sphygmomanometer to monitor the systemic circulation.

### Table 1. Baseline Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Protocol 1</th>
<th>Protocol 2</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>Heart failure</td>
</tr>
<tr>
<td>n</td>
<td>14</td>
</tr>
<tr>
<td>Men (n)</td>
<td>14</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40±20</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>61±9</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>83±8</td>
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<tr>
<td>Forearm blood flow (ml/min/100 ml FAV)</td>
<td>3.06±1.43</td>
</tr>
<tr>
<td>FAV (ml)</td>
<td>1,106±206</td>
</tr>
<tr>
<td>Forearm vascular resistance (units)</td>
<td>32±15</td>
</tr>
<tr>
<td>Plasma norepinephrine (pg/ml)</td>
<td>362±259</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml/hr)</td>
<td>2.4 (1.3–2.6)</td>
</tr>
</tbody>
</table>

FAV, forearm volume. Values are given as mean±SD or median (interquartile range).

*p<0.01 for heart failure compared with normal subjects within protocol 1 and protocol 2 separately.

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Methacholine was used to stimulate EDRF release and assess endothelium-dependent vasodilator responses. Methacholine, like acetylcholine, can also stimulate muscarinic smooth muscle receptors to produce vasoconstriction. However, in vivo and in vitro studies have demonstrated that its predominant effect is endothelium-dependent vasodilation since removal of the endothelium or pretreatment with methylene blue markedly attenuates responses to this agent.\textsuperscript{8,25,28–30} Methacholine was reconstituted on the morning of the study from a sterile lyophilized powder, passed through a 0.22 μm millipore filter, and diluted with 5% dextrose in water. Based on similar forearm studies performed in patients with hypercholesterolemia,\textsuperscript{22} three dosages of intra-arterial methacholine were used—0.3, 1.5, and 3.0 μg/min. The volume of the infusion was 0.5 or 1 ml/min.

Nitroprusside was chosen as the reference endothelium-independent vasodilator. Nitroprusside has been used as a control agent in other studies of endothelium-dependent vasodilation since, like nitric oxide, it directly activates smooth muscle guanylate cyclase.\textsuperscript{22,23,25} Although nitroprusside may produce vasodilation through hyperpolarization of vascular smooth muscle cells, this mechanism of action is less important than its effect on smooth muscle guanylate cyclase.\textsuperscript{31–35} Commercially available nitroprusside was prepared according to standard insert instructions with care taken to protect it from exposure to light. Nitroprusside was infused according to dosages of 1 and 5 μg/min. These dosages were selected to produce forearm blood flow responses that would be comparable in magnitude to the methacholine responses observed in the normal subjects. The volumetric rates were also 0.5 and 1.0 ml/min.

\textit{Protocol 1}

After catheter placement, the subjects rested quietly for at least 30 minutes before determination of baseline forearm blood flow. A second measurement was repeated after 5 minutes to ensure a stable baseline. Blood samples were then obtained for baseline plasma renin activity and plasma norepinephrine values.

To assess the effects of the 5% dextrose vehicle, determinations of forearm blood flow were obtained during vehicle infusion (1 ml/min). Forearm blood flow was measured repetitively during the 4-minute infusion and averaged over each minute. Because forearm blood flow measurements were constant between minutes 1, 2, 3, and 4, only the 4-minute data are presented for all interventions. Blood pressure from the contralateral arm and heart rate were recorded each minute to monitor systemic effects. After vehicle administration, there was a 10-minute rest period followed by recontrol measurements of forearm blood flow and blood pressure.

Methacholine was infused into the brachial artery with the same protocol used with vehicle. There was a 10–30-minute rest period between each methacholine infusion to allow the return of forearm blood flow to baseline before the start of the next infusion. The mean±SEM control values of forearm blood flow before the 0.3-, 1.5-, and 3.0-μg/min dosages were 3.37±0.41, 3.25±0.44, and 3.71±0.49 ml/min/100 ml FAV in the normal subjects and 3.41±0.29, 3.51±0.35, and 3.48±0.31 ml/min/100 ml FAV in the patients with heart failure. After the last infusion of methacholine, the catheter was removed, and the patients were re-started on their standard medications.

\textit{Protocol 2}

Protocol 2 was conducted to examine the specificity of the attenuated methacholine responses noted in the patients with heart failure in protocol 1. After baseline measurements, methacholine administration was identical to protocol 1 and served as a measure of the reproducibility of the methacholine responses. However, because the difference between groups was not dependent on dose, only the 0.3- and 1.5-μg/min dosages were given. After a 30-minute washout period, two dosages of nitroprusside were administered. There was a 10-minute rest period after the first dosage to allow forearm blood flow to return to baseline before the next dosage. The baseline forearm blood flow values were 2.48±0.17 and 2.87±0.67 ml/min/100 ml FAV before the first methacholine infusion and 2.95±0.51 and 2.50±0.45 ml/min/100 ml FAV before the first nitroprusside infusion in the normal subjects and patients with heart failure, respectively.

\textit{Neurohormonal Measurements}

Blood samples for plasma norepinephrine and plasma renin activity were analyzed by Dr. Ada Simon, Cardiovascular Biochemistry Laboratory, using a radioenzymatic technique with an 8% intra-assay variation\textsuperscript{26} and a radioimmunoassay,\textsuperscript{27} respectively.

\textit{Statistical Analyses}

Baseline characteristics of the normal subjects were compared with those of the patients with heart failure by \textit{t} tests for independent groups or by Wilcoxon rank sum tests for variables that were not normally distributed. Analyses of variance with dose as a repeated factor were used to compare the changes from baseline during the methacholine and nitroprusside infusions between the normal subjects and the patients with heart failure. The interaction term was used to determine if differences depended on dose. The same type of analysis was used to compare the methacholine responses in the patients with an idiopathic cardiomyopathy with those in the patients with ischemic heart disease. The relations between responses and baseline characteristics were examined by Pearson correlation coefficients. Data are summarized as mean±SEM except where noted. A probability value of 0.05 or less was considered significant.

\textit{Results}

Within both protocols, there were significant differences between the normal subjects and patients...
with heart failure (Table 1). The patients with heart failure were older and had higher resting heart rates. There were no significant differences between baseline forearm blood flow and forearm vascular resistance. However, the patients with heart failure demonstrated activation of neurohormonal systems, with significantly higher baseline levels of plasma norepinephrine and plasma renin activity compared with the normal subjects.

Figure 1 summarizes the individual and mean changes in forearm blood flow in response to the intra-arterial administration of methacholine in both normal subjects and patients with heart failure in protocol 1. There were only small changes in forearm blood flow observed with vehicle infusion with maximum responses of 0.60±0.13 and 0.31±0.19 ml/min/100 ml FAV in the normal subjects and patients with heart failure, respectively. The mean increases in forearm blood flow for the three dosages of methacholine in the normal subjects were 5.26±0.63, 10.05±0.63, and 13.22±0.86 ml/min/100 ml FAV. In contrast, the mean responses in the patients with heart failure were only 1.98±0.46, 5.48±0.79, and 8.53±1.53 ml/min/100 ml FAV. By analysis of variance, the difference between groups did not depend on dosage (p<0.42), and the overall response in the patients with heart failure was significantly less than that in the normal subjects (5.32±0.81 versus 9.52±0.60 ml/min/100 ml FAV; p=0.0003).

Forearm blood flow responses to methacholine and nitroprusside in protocol 2 are summarized in Figures 2A and 2B. The responses to two doses of methacholine in both the normal subjects and patients with heart failure were nearly identical to the responses noted in each respective group in protocol 1. As in protocol 1, the overall response to methacholine was significantly lower in the patients with heart failure compared with the normal subjects (3.42±0.62 versus 7.46±0.96 ml/min/100 ml forearm volume (FAV); p=0.001). The difference between the groups averaged 4.04±1.10 ml/min/100 ml FAV. Nitroprusside infusions in the normal subjects resulted in increases of forearm blood flow of 3.95±0.50 and 10.54±1.72 ml/min/100 ml FAV. These increases were very similar to the increases in forearm blood flow observed with methacholine in the normal subjects. In the patients with heart failure, the same doses of nitroprusside resulted in increases of forearm blood flow of 2.61±0.40 and 7.47±1.70 ml/min/100 ml FAV. The differences between the two groups did not depend on dose (p<0.47). However, in contrast to the methacholine responses, the overall nitroprusside responses were not significantly decreased in the patients with heart failure compared with the normal subjects (5.04±1.02 versus 7.25±1.12 ml/min/100 ml FAV; p=0.09). Furthermore, the average difference between patients with heart failure and normal sub-
jects with nitroprusside (2.20±0.71 ml/min/100 ml FAV) was significantly less than the difference between the groups with methacholine (4.04±1.10 ml/min/100 ml FAV; p=0.04).

Heart rate and blood pressure measured in the contralateral arm were monitored to confirm that the forearm blood flow responses after administration of methacholine and nitroprusside in both protocols were not due to systemic effects or activation of central reflexes. Throughout the dose ranges of vehicle, methacholine, and nitroprusside, there were no significant changes in heart rate or blood pressure in either group.

Because the methacholine responses were similar in protocol 1 and protocol 2, the responses were combined to examine factors that may be contributing to the attenuated responses observed in the patients with heart failure. Figure 3 contains the forearm blood flow responses to the two doses of methacholine in the 24 patients with heart failure divided by etiology. The overall responses in the 14 patients with ischemic heart disease were not significantly different from those of the 10 patients who had idiopathic dilated cardiomyopathy (4.04±0.64 versus 2.99±0.42 ml/min/100 ml FAV, respectively), suggesting that underlying atherosclerosis could not account for the decreased responsiveness in the patients with heart failure.

Age did not correlate with the increase in forearm blood flow during the 0.3- or 1.5-μg/min dosages of methacholine. As shown in Figure 4, the correlations for the higher dose were r=0.20 in the normal subjects and r=0.39 in the patients with heart failure (both p=NS). In addition, the responses in the six older normal subjects were not lower than those in the younger normal subjects, suggesting that age was not an important factor in limiting the methacholine response.

Other associations between baseline characteristics and the increase in forearm blood flow with the doses were examined to determine if these characteristics were related to the attenuated methacholine response in heart failure. In both normal subjects and patients with heart failure, there were no significant correlations between the methacholine responses and baseline forearm vascular resistance, plasma norepinephrine, or plasma renin activity.

Discussion

The present study demonstrated that forearm vasodilator responses to subsystemic doses of intrarrenal methacholine, an endothelium-dependent vasodilator, are reduced in patients with heart failure compared with normal subjects. The decreased methacholine responses were not related to age, presence of underlying ischemic heart disease, baseline forearm vascular resistance, plasma norepinephrine, or plasma renin activity. This impairment is out of proportion to the mildly impaired response to nitroprusside, an endothelium-independent vasodilator. Therefore, these data suggest that endothelium-dependent vasodilation is attenuated in patients with heart failure.

There are increasing data from animal experiments demonstrating that certain disease states characterized by vasoconstriction and/or vascular pathology, including hypertension and atherosclerosis, are associated with decreased endothelium-dependent vasodilation. Studies in humans have revealed paradoxical vasoconstrictor responses to the intracoronary administration of acetylcholine in patients with atherosclerosis and decreased forearm vasodilator responses to endothelium-dependent va-
Sodilators in patients with hypercholesterolemia and hypertension.\textsuperscript{22,23,25} Studies in heart failure have been limited. One recent study in an animal model demonstrated abnormalities in endothelium-dependent vasodilation in the femoral artery.\textsuperscript{16} In patients with dilated cardiomyopathy, coronary vasodilation induced by acetylcholine is markedly impaired in contrast to the mild reduction in coronary vasodilation induced by papaverine, an endothelium-independent vasodilator.\textsuperscript{40} The present study extends these observations by demonstrating abnormalities in endothelium-dependent vasodilation in the peripheral vasculature in patients with heart failure.

Patients with heart failure are frequently characterized by heightened vasoconstriction and reduced vasodilator responses to reactive hyperemia.\textsuperscript{2,41} This baseline vasoconstriction may explain in part the slightly but not significantly reduced responses to intra-arterial nitroprusside noted in protocol 2. However, two findings suggest that there is a specific abnormality in endothelium-dependent vasodilation in heart failure in addition to nonspecific vasoconstriction. First, the impairment of methacholine responses in the present study was out of proportion to the attenuation observed with nitroprusside. The methacholine response was highly reproducible between two separate patient groups in protocols 1 and 2 and is unlikely to be related to patient selection. Second, other studies evaluating forearm vasodilator responses to endothelium-independent agents in patients with heart failure, including nitroprusside,\textsuperscript{42,43} isoproterenol,\textsuperscript{42} and yohimbine,\textsuperscript{27} have not demonstrated significant differences compared with normal subjects.

The mechanisms of the decreased endothelium-dependent responses in patients with heart failure are not known. Although the presence of atherosclerosis may be an important factor,\textsuperscript{21,22,38} we did not demonstrate any effect of underlying ischemic heart disease. Furthermore, the brachial artery and the forearm circulation are only rarely involved in atherosclerosis. Increasing age has also been shown to reduce endothelium-dependent vasodilation.\textsuperscript{38} However, the association with age has been modest ($r = -0.44$),\textsuperscript{38} and there was no association with age in the present study. The decreased responses may also be due to increased vasoconstriction mediated in part by higher levels of plasma norepinephrine and plasma renin activity that nonspecifically oppose the vasodilator effects of methacholine.\textsuperscript{44} However, the methacholine responses in heart failure did not correlate with levels of either neurohormone. Furthermore, the average difference with methacholine was significantly greater than the average difference with nitroprusside in the same patients. It is possible that medications taken by the patients may have affected the results. All patients were treated with digoxin, which may inhibit endothelium-dependent relaxation through its effect on hyperpolarizing factors.\textsuperscript{45} This effect is probably less important in vivo since Treasure et al.\textsuperscript{40} found no difference in endothelium-dependent coronary vasodilation between patients on and those off digoxin.\textsuperscript{40} In addition, in this study, there were no differences in the responses in the patients previously taking converting enzyme inhibitors or nitrates. It is also possible that methacholine responses may be different among patients with different New York Heart Association functional classification. Therefore, additional studies, including patients from all four functional classes, as well as controlled studies with vasodilating drugs are necessary. Finally, because heart failure is associated with increased interstitial edema, the reduced methacholine responses could be related to impaired diffusion of EDRF from the endothelium to the smooth muscle. However, if this were the case, one would expect a similar defect in vasodilation to nitroprusside because it must diffuse from the intravascular compartment through the endothelium to activate smooth muscle guanylate cyclase.

Although the reduced methacholine responses in the patients with heart failure have been interpreted as demonstrating impaired endothelium-dependent vasodilation, there are alternative explanations. Methacholine has a direct smooth muscle constrictor effect as well as an inhibitory effect on norepinephrine release from sympathetic nerve endings and can stimulate prostacyclin production. However, other studies using the same forearm model in patients with hypertension or hypercholesterolemia have demonstrated that forearm vasodilation with acetylcholine was not changed by phentolamine or acetylsalicylic acid.\textsuperscript{22,23,25} suggesting that the vascular effects of acetylcholine were not mediated by effects on catecholamine release or stimulation of vasodilating prostaglandins. Furthermore, forearm vasodilator responses to acetylcholine are inhibited by L-NMMA, a false substrate for nitric oxide.\textsuperscript{46} However, additional studies of other endothelium-dependent vasodilators are needed to confirm the abnormal endothelial function in heart failure.

It should be emphasized that the present study is limited to stimulated endothelium-dependent vasodilation and changes in flow. Therefore, additional studies using blockers of EDRF are necessary to provide data concerning basal release of EDRF and contributions to basal arteriolar tone. In addition, because changes in flow are mediated by effects on small resistance vessels, other studies measuring changes in compliance and diameter are necessary to characterize endothelial function of the larger arteries of patients with heart failure. Finally, this study is limited by the fixed order of drug administration (methacholine followed by nitroprusside), which may have affected the results.

The significance of the present finding relates to the possibility that this local mechanism may serve to modulate vasoconstriction mediated by circulating neurohormonal messages and that an abnormal local vasodilator response may contribute to heightened vasoconstriction that is characteristic of heart failure. It is also possible that regional differences in endo-
Endothelium-dependent vasodilation may explain in part the differences in redistribution of cardiac output at rest and during exercise and that the benefits of systemic vasodilator therapy may be mediated by their effects on restoring endothelium-dependent vasodilation, but additional study is required. Overall, however, these data suggest that a focus toward local mechanisms of vasomotor regulation in addition to the previous emphasis on circulating neurotransmitters may further our understanding of the syndrome of heart failure.

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