Impaired endothelium-mediated vasodilation is not the principal cause of vasoconstriction in heart failure

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Negrao, Carlos E., Michele A. Hamilton, Gregg C. Fonarow, Antoine Hage, Jaime D. Moriguchi, and Holly R. Middlekauff. Impaired endothelium-mediated vasodilation is not the principal cause of vasoconstriction in heart failure. Am. J. Physiol. Heart Circ. Physiol. 278: H168–H174, 2000.—The extent to which abnormal endothelium-dependent vasodilator mechanisms contribute to abnormal resting vasoconstriction and blunted reflex vasodilation in heart failure is unknown. The purpose of this study was to test the hypothesis that the resting and reflex abnormalities in vascular tone that characterize heart failure are mediated by abnormal endothelium-mediated mechanisms. Thirteen advanced heart-failure patients (New York Heart Association III-IV) and 13 age-matched normal controls were studied. Saline, acetylcholine (20 µg/min), or L-arginine (10 mg/min) was infused into the brachial artery, and forearm blood flow was measured by venous plethysmography at rest and during mental stress. At rest, acetylcholine decreased forearm vascular resistance in normal subjects, but this response was blunted in heart failure. During mental stress with intra-arterial acetylcholine or L-arginine, the decrease in forearm vascular resistance was not greater than during saline control in heart failure (saline control vs. acetylcholine (7 ± 3 vs. 6 ± 3, P = NS) or vs. L-arginine (9 ± 2 units, P = NS)). The increase in forearm blood flow was not greater than during saline control in heart failure (saline control vs. acetylcholine (1.2 ± 0.3 vs. 1.3 ± 0.3, P = NS), or vs. L-arginine (1.2 ± 0.2 ml·min⁻¹·100 ml⁻¹, P = NS)). Furthermore, during mental stress with nitroprusside, the decrease in forearm vascular resistance was not greater than during saline control (saline control vs. nitroprusside (7 ± 3 vs. 5 ± 4 ml·min⁻¹·100 g⁻¹, P = NS)), and the increase in forearm blood flow was not greater than during saline control (saline control vs. nitroprusside (1.2 ± 0.3 vs. 1.3 ± 0.5 ml·min⁻¹·100 g⁻¹, P = NS)). Because the endothelial-independent agent nitroprusside was unable to restore resting and reflex vasodilation to normal in heart failure, we conclude that impaired endothelium-mediated vasodilation with acetylcholine-nitric oxide cannot be the principal cause of the attenuated resting- or reflex-mediated vasodilation in heart failure.

HEART failure is characterized by resting vasoconstriction and abnormal vasodilatory responses during mental and physical stress (4, 6, 7, 10, 16, 17). This abnormal vasomotor tone may be due to increased vasoconstrictor influences and/or decreased vasodilator influences. In this study we investigated the contribution of the vasodilatory mechanisms to the abnormal resting and reflex vasomotor tone in heart failure. There is pharmacological evidence for impaired endothelial-dependent vasodilation in heart failure, mediated by endothelium-dependent cholinergic and nitric oxide mechanisms (4, 6, 7). Furthermore, actual physiological reflex vasodilator responses, which depend on nitric oxide, are blunted in heart failure (10). Kubo and colleagues (7) administered intra-arterial methacholine, a muscarinic agent, and nitroprusside, an endothelium-independent vasodilator, in heart-failure patients and normal controls. The vasodilatory responses to methacholine were significantly more blunted compared with vasodilatory responses to nitroprusside in heart-failure patients compared with normal controls, consistent with abnormal cholinergic vasodilatory mechanisms in heart failure (7). Hirooka and colleagues (4) administered intra-arterial L-arginine, a precursor of endothelium-derived nitric oxide, in heart-failure patients during simultaneous acetylcholine infusion and found the attenuated cholinergic vasodilation was improved. Both of these studies in humans with heart failure are consistent with abnormal endothelium-dependent vasodilation, mediated by acetylcholine-nitric oxide mechanisms.

In addition to abnormal resting vasoconstriction, reflex vasodilation in heart failure is attenuated. For example, during mental stress in heart failure, reflex forearm vasodilation is blunted (10). In animals and normal humans, this reflex vasodilation during mental stress has been shown to be mediated by the endothelium-dependent mechanisms, acetylcholine and nitric oxide (2, 8). The extent to which abnormal endothelium-dependent vasodilator mechanisms in heart failure contribute to the abnormal resting vasoconstriction and blunted reflex vasodilation is unknown. The purpose of this study was to test the hypothesis that the resting and reflex abnormalities in vasoconstriction that characterize heart failure are mediated by abnormal endothelium-mediated mechanisms. We studied the vasodilatory responses during intra-arterial administration of the endothelium-dependent agents acetylcholine and L-arginine and the endothelium-indepen-
dent agent nitroprusside at rest and during mental stress, which activates endothelium-mediated mechanisms.

METHODS

Study Population

After informed written consent, 13 advanced congestive heart-failure patients (mean age 49 ± 12 yr) and 13 age-matched normal controls (mean age 49 ± 15 yr, P = NS) participated in two of three drug protocols. Thirteen heart-failure patients and 13 normal controls were enrolled in the study of saline and acetylcholine. Eight heart-failure patients and eight normal controls were enrolled in the study of saline and L-arginine infusion during mental stress. Five heart-failure patients and five normal controls were enrolled in the study of saline and sodium nitroprusside. The study protocols were approved by the University of California Los Angeles (UCLA) Human Subject Protection Committee. Normal subjects were healthy as confirmed by medical history and physical examination and were not taking medications. In the heart-failure patients, the etiology of heart failure was coronary artery disease in eight and idiopathic dilated cardiomyopathy in five patients. Medications, including vasodilators, diuretics, and digoxin, were discontinued 24–36 h before the study protocol under medical supervision in the UCLA Clinical Research Center. All patients had advanced (New York Heart Association, functional class III-IV) congestive heart failure. As measured by echocardiography, quantified mean left ventricular ejection fraction was 0.20 ± 0.03 and peak oxygen consumption was 13.9 ± 1.5 ml/kg. Patients and normal controls abstained from caffeine for 24 h before the study. The studies were performed in the postabsorptive state.

Measurements and Procedures

Forearm blood flow. Forearm blood flow was measured by means of venous occlusion plethysmography. The nondominant arm was elevated just above heart level to ensure adequate venous drainage. A mercury-filled Silastic tube attached to a low-pressure transducer was placed around the forearm and connected to a plethysmograph (Hokanson, Bellevue, WA). Sphygmomanometer cuffs were placed around the wrist and upper arm. The wrist cuff was inflated to suprasystolic level for 1 min prior to the flow measurements. At 15-s intervals, the upper cuff inflated above venous pressure for 7–8 s. The rate of increase in strain reflected the rate of increase in forearm volume and arterial blood flow. Forearm vascular resistance (units) was calculated by dividing mean arterial pressure (mmHg) by forearm blood flow (ml·min⁻¹·100 ml tissue⁻¹).

Drug infusions. Acetylcholine (CIBA) was used under IND-50695 and diluted in normal saline (40 µg/ml) just before the study. L-Arginine (Pharmacia) was used under IND-50695 and diluted in normal saline (100 mg/ml). Sodium nitroprusside (Gensia Laboratories) was diluted in normal saline (0.8 µg/ml).

Mental stress testing. Mental stress was elicited by the Stroop color-word test and mental arithmetic (12, 13). During the Stroop color-word test, subjects were shown a series of names of colors written in a different color ink from the color specified. The subject was asked to identify the color of the ink, not read the word. In the verbally administered mental arithmetic test, subjects were asked to rapidly subtract a one- or two-digit number from a three- or four-digit number. The difficulty of the task was tailored to the ability of the subject. The subject was urged to proceed as rapidly as possible and was gently chastised for incorrect responses. The subject was asked to try his best, but the number of correct answers was not quantified. Each subject was asked to assess task difficulty on completion of the protocol, using a standard five-point scale (1): 0, not stressful; 1, somewhat stressful; 2, stressful; 3, very stressful; 4, very, very stressful.

Mental stress testing for 4 min using the same mental stress test as in the saline-control study was performed. Following the mental stress test, the recovery values were recorded for 4 min simultaneously with drug infusion and then hemodynamics were allowed to return to baseline. Arterial pressure and heart rate were recorded for 2 min. Four minutes of mental stress testing were then begun. After the mental stress test, the recovery values were recorded for 4 min followed by a rest period to allow hemodynamics to return to baseline. After a 15-min rest period, saline was infused (0.8 ml/min, saline control) and baseline values for forearm blood flow, arterial pressure, and heart rate were recorded for 2 min. Four minutes of mental stress testing were then begun. After the mental stress test, the recovery values were recorded for 4 min followed by a rest period to allow hemodynamics to return to baseline. After a 15-min rest period, intra-arterial infusion of acetylcholine (20 µg/min), L-arginine (10 mg/min), or nitroprusside (0.8 µg/min) was started. After 4 min, a new baseline forearm blood flow, arterial pressure, and heart rate were recorded. Mental stress testing for 4 min using the same mental stress test as in the saline-control study was performed. Following the mental stress test, the recovery values were recorded for 4 min simultaneously with drug infusion and then hemodynamics were allowed to return to baseline. Arterial pressure and heart rate were recorded for 2 min. Four minutes of mental stress testing were then begun. After the mental stress test, the recovery values were recorded for 4 min followed by a rest period to allow hemodynamics to return to baseline. Arterial pressure and heart rate were recorded for 4 min. Following the mental stress test, the recovery values were recorded for 4 min simultaneously with drug infusion and then hemodynamics were allowed to return to baseline. Arterial pressure and heart rate were recorded for 2 min. Four minutes of mental stress testing were then begun. After the mental stress test, the recovery values were recorded for 4 min followed by a rest period to allow hemodynamics to return to baseline. Arterial pressure and heart rate were recorded for 4 min. Following the mental stress test, the recovery values were recorded for 4 min simultaneously with drug infusion and then hemodynamics were allowed to return to baseline. Arterial pressure and heart rate were recorded for 2 min. Four minutes of mental stress testing were then begun. After the mental stress test, the recovery values were recorded for 4 min followed by a rest period to allow hemodynamics to return to baseline. Arterial pressure and heart rate were recorded for 4 min. Following the mental stress test, the recovery values were recorded for 4 min simultaneously with drug infusion and then hemodynamics were allowed to return to baseline. Arterial pressure and heart rate were recorded for 2 min. Four minutes of mental stress testing were then begun. After the mental stress test, the recovery values were recorded for 4 min followed by a rest period to allow hemodynamics to return to baseline. Arterial pressure and heart rate were recorded for 4 min.

Statistical Analysis

Statistical analysis was performed by using paired and unpaired Student's t-tests and two-group repeated measure ANOVA. The data are presented as means ± SE, mean absolute change, or mean absolute value unless otherwise stated. Probability values of <0.05 were considered statistically significant.

![Fig. 1. Timeline of experimental protocol (see Experimental Protocol for explanation).](http://ajpheart.physiology.org/)
Hemodynamic Responses to Acetylcholine, L-Arginine, and Sodium Nitroprusside During Mental Stress

The baseline data before mental stress testing are shown in Table 1. Heart rate was significantly greater in heart-failure patients when compared with normal controls, but mean arterial pressure was not significantly different between the two groups. Baseline forearm blood flow was significantly lower and baseline forearm vascular resistance was significantly greater in patients with heart failure when compared with normal controls.

Perceived Difficulty of Mental Stress Testing

The perceived difficulty of each saline-control mental stress test was not different from that of the mental stress test performed during drug infusion in either heart-failure patients or normal controls. Similarly, perceived difficulty of mental stress testing was not different between normal subjects and heart-failure subjects.

Hemodynamic Responses to Mental Stress With Saline Infusion

Forearm blood flow increased significantly in both normal controls and heart-failure patients, although the increase was significantly greater in normal controls. During mental stress forearm vascular resistance decreased in both normal controls and heart-failure patients.

Hemodynamic Responses to Acetylcholine, L-Arginine, and Sodium Nitroprusside at Rest

Resting heart rate and arterial pressure were not changed by acetylcholine, L-arginine, or sodium nitroprusside. Acetylcholine infusion significantly increased resting values of forearm blood flow in both normal controls and heart-failure patients, although the increase was significantly blunted in heart-failure patients.

Hemodynamic Responses to Acetylcholine, L-Arginine, and Sodium Nitroprusside at Rest

Forearm vascular resistance remained blunted during L-arginine administration. Similarly, during sodium nitroprusside at rest, the increase in forearm blood flow was significantly blunted in heart-failure patients compared with normal subjects. During sodium nitroprusside at rest, the increase in forearm blood flow was significantly blunted in heart-failure patients.

DISCUSSION

Abnormal resting vasoconstriction and blunted reflex vasodilation in heart failure may be due to increased vasoconstrictor mechanisms, decreased vasodilatory mechanisms, or both. In this study we focused on the vasodilatory mechanisms. The main findings of the present investigation are that 1) at rest vasodilation during administration of endothelium-dependent and -independent agents acetylcholine and nitroprusside is
blunted in heart-failure patients compared with controls; 2) in heart-failure patients reflex vasodilation during mental stress, which is normally mediated by endothelial-dependent mechanisms, is not restored by the endothelium-dependent agent acetylcholine; 3) L-arginine, the substrate for nitric oxide production, does not restore reflex vasodilation during mental stress; and 4) reflex vasodilation during mental stress in heart-failure patients is not restored by the administration of the endothelium-independent agent sodium nitroprusside. Based on these findings, we conclude that impaired acetylcholine-nitric oxide mechanisms are not the principal cause of the attenuated resting or reflex-mediated vasodilation in heart failure. These findings support the concept that heightened vasoconstrictor influences, such as increased central sympathetic neural outflow, angiotensin, and endothelin activity, underlie the abnormalities of resting and reflex vascular tone in heart failure (9, 15).

Acetylcholine may be released by activation of “cholinergic nerves” or by flow-induced activation of endothelial cells. In normal subjects during mental stress, sympathetic withdrawal at the initiation of mental stress has been described, thereby leading to flow-induced acetylcholine release and nitric oxide activation (3). In heart failure, which is characterized by heightened sympathetic nerve activity, sympathetic nerve withdrawal may not occur during mental stress or may be inadequate to activate flow-induced acetylcholine-nitric oxide release.

In our study, we administered exogenous acetylcholine to determine whether, in the presence of vasodilatory concentrations of acetylcholine, vasodilatory mechanisms would be restored in heart failure. Acetylcholine administration at rest significantly increased baseline forearm blood flow in both heart-failure patients and normal controls, but this increase was blunted in heart-failure patients. Blunted muscle vasodilatory responses to acetylcholine in patients with dilated cardiomyopathy at rest has been observed by others as well (6). During mental stress, acetylcholine failed to restore the vasodilatory responses to normal in heart-failure patients. This blunted vasodilatory response to acetylcholine infusion suggests that the muscle vasodilatory dysfunction in heart-failure patients is not solely attributable to an impairment in the cholinergic activation. Other potential mechanisms of the impaired muscle vascular response to local intra-arterial infusion of acetylcholine may include muscarinic receptor dysfunction. The density of muscarinic receptors in the myocardium is reduced in dogs with chronic ventricular pressure overload (14) and may be reduced in the vasculature as well. Alternatively, abnormal endothelial synthesis of nitric oxide in response to cholinergic activation may underlie the abnormal vasodilatation during mental stress in heart failure. To further evaluate the contribution of insufficient nitric oxide synthesis at rest and during mental stress in heart failure, we infused L-arginine intra-arterially.

In humans with heart failure, acute intra-arterial administration of L-arginine (10 mg/min) has been shown to enhance vasodilation during acetylcholine-mediated vasodilation, consistent with defective endothelial function in heart failure (4). In our study,
L-arginine (10 mg/min) had no effect on forearm blood flow responses at rest. Furthermore, L-arginine did not restore the vasodilatory responses during mental stress in heart-failure patients to normal. These findings suggest that the blunted muscle vasodilation at rest and in response to mental stress in heart-failure patients is not due to the lack of substrate for nitric oxide production. In heart failure, nitric oxide-mediated vasodilation may not be limited by substrate availability for enzymatic conversion to nitric oxide but may be limited by enzyme availability. Nitric oxide synthase expression is downregulated in low-flow states. This is likely the case in heart failure, in which blood flow is decreased (9, 15). Alternatively, abnormal end-organ response to nitric oxide release, in other words, endothelial-independent mechanisms, may underlie the abnormal vasodilatory response to mental stress in heart failure.

To assess the integrity of endothelial-independent vasodilation in these heart-failure patients, sodium nitroprusside was administered intra-arterially. The increase in resting forearm blood flow during administration of sodium nitroprusside was blunted in heart-failure patients compared with normal controls. Similar findings have been previously reported by other investigators (6) but not all (4). Nitroprusside failed to restore the vasodilatory response during mental stress to normal in heart-failure patients. These findings are consistent with heightened basal muscle vasoconstriction in heart-failure patients, which cannot be offset by the exogenous, endothelial-independent agent nitroprusside. Furthermore, because endothelial-independent vasodilatory mechanisms are abnormal in heart failure, we cannot implicate abnormalities of cholinergic neural activation or nitric oxide release as the principal mechanism of the abnormal vasodilation at rest or in response to mental stress in heart failure. The abnormal tonic vasoconstriction in heart failure is likely multifactorial, including endothelin, angiotensin, and sympathetic neural vasoconstrictor activity (9, 15), as well as structural vascular changes which limit vasodilatory capacity (16, 17).

Limitations

Intra-arterial infusion of saline or drugs could alter the vasodilatory responses during mental stress in a volume-dependent manner. Previous studies (4, 5) have demonstrated that intra-arterial infusion of saline at 0.6–0.8 ml/min does not alter forearm blood flow. In the present study, the maximal volume infused for saline control or drugs was 0.8 ml/min. Therefore, the risk of volume-dependent changes in forearm blood flow was minimized.

We did not evaluate the changes in forearm blood flow at rest or during mental stress during simulta-
neous administration of acetylcholine and L-arginine. We reasoned that if both systems were abnormal, the simplest way to study the contribution of each was by separate infusions. Similarly, if only one system were abnormal, this would be easiest to determine during separate infusions. In our study, we found no effect with L-arginine alone in heart-failure patients. However, because we did not see any L-arginine effect in the normal controls, we are unable to invoke a role for abnormal nitric oxide synthesis in the blunted vasodilatory response to mental stress in heart failure.

Resting values of forearm vascular resistance were significantly higher in heart-failure patients compared with normal controls, which could complicate interpretation of changes in vasomotor tone during experimental protocols. In animal studies, the importance of the initial resistance on the magnitude of response to a vasodilator stimulus is significant (11). That is, an elevation in baseline vascular resistance may lead to a nonspecific enhancement of vasomotor responses to a vasodilator stimuli. Thus we would expect this difference in baseline forearm vascular resistance between heart-failure patients and normal controls to lead to a nonspecific exaggeration in vasodilatory responses in heart failure. Of course, this did not occur, and in fact the vasodilatory responses in heart-failure patients were significantly blunted during drug infusions and mental stress testing.

It is possible that accelerated degradation, rather than inadequate production, of nitric oxide contributes to the increased vasoconstriction characteristic of heart failure. However, if this were the major cause of vasoconstriction in heart failure, we would have expected that the pharmacological dose of nitroprusside used in this study to have overcome degradation by endogenous substances and restore the vasodilatory response during mental stress to normal.

In summary, resting muscle blood flow is reduced and reflex vasodilatory responses during mental stress are blunted in patients with heart failure. Forearm intra-arterial administration of acetylcholine or L-arginine failed to restore to normal the vasomotor tone at rest or in response to mental stress in patients with heart failure. Sodium nitroprusside did not restore the vasomotor tone at rest or in response to mental stress in heart failure, consistent with a heightened basal vasoconstriction in heart failure. Altered cholinergic and/or nitric oxide pathways may be contributory, but are insufficient to explain the persistent resting vasoconstriction and blunted reflex vasodilatory responses in patients with heart failure.

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