

Sympathetic activation restrains endothelium-mediated muscle vasodilatation in heart failure patients

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Submitted 8 December 2004; accepted in final form 11 March 2005

Santos, Amilton C., Maria J. N. N. Alves, Maria U. P. B. Rondon, Antonio C. P. Barretto, Holly R. Middlekauff, and Carlos E. Negrão. Sympathetic activation restrains endothelium-mediated muscle vasodilatation in heart failure patients. *Am J Physiol Heart Circ Physiol* 289: H593–H599, 2005. First published March 18, 2005; doi:10.1152/ajpheart.01240.2004.—Although the vasodilatory response during mental stress is blunted in heart failure (HF), the mechanisms underlying this phenomenon are not fully understood. We tested the hypothesis that sympathetic activity limits the endothelium-dependent vasodilatation during mental stress in chronic HF patients. Twenty-one HF patients (age 45 ± 2 yr, functional classes III and IV, New York Heart Association) and 22 age-matched normal controls (NC; age 42 ± 2 yr, $P = 0.13$) were studied at rest and during 4 min of Stroop color-word test with brachial intra-arterial saline, acetylcholine (endothelium dependent), phentolamine (α -blocker), and phentolamine plus acetylcholine infusion. Forearm blood flow was measured by venous occlusion plethysmography. Baseline forearm vascular conductance (FVC) was significantly lower in HF patients (2.18 ± 0.12 vs. 3.66 ± 0.22 units, $P = 0.001$). During mental stress with saline, the changes in FVC were significantly blunted in HF patients compared with NC (0.92 ± 0.20 vs. 2.13 ± 0.39 units, $P = 0.001$). In HF, the vasodilatation with acetylcholine was similar to saline control and significantly lower than in NC. In HF patients, phentolamine significantly increased FVC responses (1.16 ± 0.20 vs. 2.09 ± 0.29 units, $P = 0.001$), and the difference between HF patients and NC tended to decrease (2.09 ± 0.29 vs. 3.61 ± 0.74 units, $P = 0.052$). The vasodilatation with phentolamine plus acetylcholine was similar between HF and NC (4.23 ± 0.73 vs. 4.76 ± 1.03 units, $P = 0.84$). In conclusion, sympathetic activation mediates the blunted muscle endothelium-mediated vasodilatation during mental stress in HF patients.

mental stress; forearm vasodilatation; endothelial function

HEART FAILURE is characterized by systemic vasoconstriction. The increase in resting cortical renal vascular resistance and forearm vascular resistance has been well characterized in patients with advanced heart failure (9, 11, 17). This excessive resting vasoconstriction is present during physiological reflex responses as well. The forearm vasodilatation during mental stress and exercise is impaired in chronic heart failure (10, 11). However, the mechanisms underlying this blunted vasodilatory response remain little understood.

In normal individuals, mental stress provokes reflex forearm vasodilatation (3), which has been largely attributed to nitric oxide production by means of β_2 -adrenoreceptor and muscarinic recep-

tor stimulation (3). A previous study (4) showed that treatment with phentolamine and bretylium to eliminate the α -adrenergically mediated vasoconstriction markedly increased the forearm vasodilatory response during mental stress in healthy humans. Thus, in normal individuals, sympathetic activation restrains endothelium-mediated vasodilatation during mental stress.

To investigate the mechanisms involved in the blunted muscle vasodilatation at rest and during reflex perturbations in heart failure, Hirooka and colleagues (6) administered acetylcholine and L-arginine into the brachial artery at rest and during reactive hyperemia in heart failure patients. In contrast to normal control individuals, acetylcholine did not increase forearm blood flow in heart failure patients. The combination of acetylcholine with L-arginine, however, significantly increased muscle blood flow in heart failure patients (6, 15). These findings were interpreted to mean that the blunted vasodilatation in chronic heart failure was attributable to endothelial dysfunction. However, endothelial dysfunction may not explain the blunted vasodilatation in heart failure during mental stress, because we (10) found that an intrabrachial administration of acetylcholine and L-arginine did not restore the muscle vasodilatation during mental stress in chronic heart failure patients. Muscle vasodilatation depends on the balance of vasodilator forces and vasoconstrictor forces. Thus we reasoned that the impaired muscle vasodilatation during mental stress could be a consequence of endothelial dysfunction and/or, alternatively, excessive muscle sympathetic nerve activity. In a previous study (9), the absolute muscle sympathetic nerve activity levels during mental stress were significantly increased in heart failure patients compared with normal controls. Thus the purpose of this study was to test the hypothesis that the blunted vasodilatation during mental stress in heart failure is due to this excessive sympathetic vasoconstrictor activity.

We used pharmacological strategies to blunt sympathetic vasoconstriction (phentolamine) and to activate endothelium-dependent vasodilatory mechanisms (acetylcholine). We found that blockade of α -adrenergic vasoconstrictor activity restored endothelium-mediated vasodilatation during mental stress in patients with heart failure.

METHODS

Study Population

After informed consent was obtained, 21 chronic heart failure patients (45 ± 2 yr) and 22 age-matched normal controls (42 ± 2 yr,

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$P = 0.13$) participated in the study. The study protocol was approved by the Human Subject Protection Committees of the Heart Institute [Instituto do Coração (InCor)] and Clinical Hospital, University of Sao Paulo Medical School. 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 patients, idiopathic dilated cardiomyopathy in six patients, and Chagas disease in seven patients. All patients were taking vasodilators, diuretics, and digoxin, which were discontinued 12 h before the study under medical supervision in the Heart Institute (InCor), University of Sao Paulo Medical School. Because patients were enrolled before β -blockade became standard therapy in heart failure, no patient was taking β -blocker medication. All patients had advanced heart failure (New York Heart Association functional classes III and IV). As measured by echocardiography, the quantified mean left ventricular ejection fraction was 0.27 ± 0.02 . Patients and normal controls abstained from caffeine before the study. The studies were performed in the postabsorptive state.

Measurements and Procedures

Forearm blood flow. Forearm blood flow was measured by venous occlusion plethysmography. The nondominant arm was elevated 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 plethysmography (Hokanson; Bellevue, WA). Sphygmomanometer cuffs were placed around the wrist and upper arm. At 15-s intervals, the upper cuff was inflated above venous pressure for 7–8 s. When forearm blood flow was measured, flow to the hand was excluded by inflating the wrist cuff to suprasystolic pressure (250 mmHg) (4). Forearm blood flow ($\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml tissue}^{-1}$) was determined on the basis of a minimum of four separate readings. Forearm vascular conductance (units) was calculated as $(\text{forearm blood flow})/(\text{mean blood pressure}) \times 100$ and was expressed in “units” [$100 \text{ ml (dl of tissue)}^{-1} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$], as previously reported (16).

Mental stress testing. Mental stress was elicited by the Stroop color-word test (13). During the Stroop color-word test, subjects were shown a series of names of colors written in different color ink from the color specified. The subjects were asked to identify the color of the ink, not read the word. Each patient was asked to assess task difficulty on completion of the protocol, using a standard five-point scale (2): 0, not stressful; 1, somewhat stressful; 2, stressful; 3, very stressful; and 4, very, very stressful.

Miscellaneous measurements. Arterial pressure was invasively directly monitored by an intra-arterial catheter. Heart rate was monitored continuously through lead II of the electrocardiogram.

Experimental Protocols

Protocol 1: forearm blood flow during mental stress under cholinergic/nitric oxide stimulation. Eight patients with heart failure and eight normal controls took part in this protocol (Fig. 1). The purpose of this protocol was to test the endothelium-dependent muscle vasodilatory response during mental stress in heart failure patients. The subject was positioned, and electrocardiogram leads were placed on the chest. An intra-arterial catheter was placed into the brachial arterial of the nondominant arm. The catheter was attached to a three-way stopcock for ease of intra-arterial drug infusion. Cuffs for forearm blood flow measurements were placed on the nondominant arm. After a 15-min rest period, saline was infused (0.5 ml/min), and baseline values for forearm blood flow, arterial pressure, and heart rate were recorded for 3 min. Four minutes of mental stress test were then begun. After a 15-min rest period, an intra-arterial infusion of acetylcholine (40 $\mu\text{g}/\text{min}$) was started (4, 7). After 5 min, a new baseline forearm blood flow, arterial pressure, and heart rate were recorded. Mental stress test was performed for 4 min simultaneously with acetylcholine infusion.

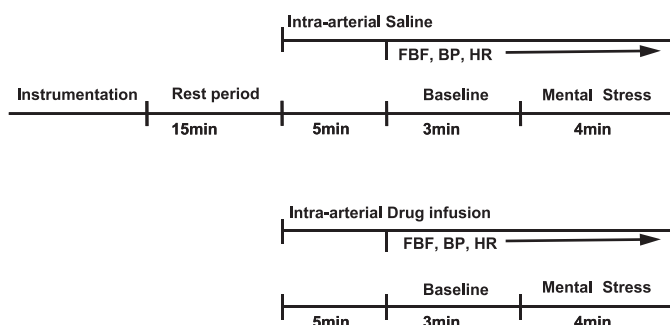


Fig. 1. Timeline of experimental protocols (see *Experimental Protocols* for an explanation). FBF, forearm blood flow; BP, blood pressure; HR, heart rate.

Protocol 2: forearm blood flow during mental stress under α_1 -adrenergic blockade and cholinergic/nitric oxide stimulation. Eleven patients with heart failure and thirteen normal controls took part in this protocol (Fig. 1). The purpose of this protocol was to determine whether sympathetic nerve activity restrains the endothelium-dependent muscle vasodilatory response during mental stress in heart failure patients. After a 15-min rest period after instrumentation as in *protocol 1*, saline was infused (0.5 ml/min), and baseline values for forearm blood flow, arterial pressure, and heart rate were recorded for 3 min. Four minutes of mental stress test were then begun. After a 15-min rest period, an intra-arterial infusion of phentolamine (100 $\mu\text{g}/\text{min}$) was started (4). After 5 min, a new baseline forearm blood flow, arterial pressure, and heart rate were recorded. The mental stress test was performed for 4 min simultaneously with a phentolamine infusion. After a 15-min rest period, an intra-arterial infusion of phentolamine (100 $\mu\text{g}/\text{min}$) associated with acetylcholine (40 $\mu\text{g}/\text{min}$) was started. After 5 min, a new baseline forearm blood flow, arterial pressure, and heart rate were recorded, followed by a 4-min period of mental stress test with phentolamine and acetylcholine infusion.

Statistical Analysis

Possible baseline differences between groups were tested by unpaired *t*-test. Two-way ANOVA with repeated measures was performed to test the resting differences within groups and between groups with saline infusion and drug infusion. In addition, two-way ANOVA with repeated measures was performed to test the differences within groups and between groups during mental stress. When a significant difference was found, Scheffé's post hoc comparison was performed. Data are presented as means \pm SE. Probability values of $P < 0.05$ were considered statistically significant.

RESULTS

Basal Measurements

Basal measurements are presented in Table 1. Baseline mean blood pressure was not different between normal controls and heart failure patients. Baseline heart rate was significantly greater in heart failure patients compared with normal controls. Both baseline forearm blood flow and baseline forearm vascular conductance were significantly lower in heart failure patients compared with normal controls.

Hemodynamic Responses to Mental Stress With Saline Control

During mental stress with saline control infusion, no significant difference in the levels of exertion perception during mental stress in heart failure patients and normal controls were found (1.75 ± 0.18 vs. 2.15 ± 0.19 , $P = 0.14$). These findings

Table 1. Baseline hemodynamics

	NC (n = 22)	HF (n = 21)	P Value
MBP, mmHg	76±2	71±2	NS
HR, beats/min	68±1	81±3	0.001
FBF, ml·min ⁻¹ ·100 ml ⁻¹	2.75±0.15	1.54±0.10	0.001
FVC, units	3.66±0.22	2.18±0.12	0.001

Values are means ± SE; n, no. of subjects. NC, normal controls; HF, heart failure; MBP, mean blood pressure; HR, heart rate; FBF, forearm blood flow; FVC, forearm vascular conductance; NS, not significant.

imply that the perception of stress during the Stroop color-word test were similar between heart failure patients and normal controls.

Hemodynamic responses to mental stress with saline are shown in Table 2. Heart rate and mean blood pressure increased during mental stress in both heart failure patients and normal controls. During protocol 1, under cholinergic/nitric

Table 2. HR and MBP responses during mental stress with intra-arterial infusion of saline, phentolamine, phentolamine plus acetylcholine, and acetylcholine in NC and HF patients

	Baseline	Infusion			
		1 min	2 min	3 min	4 min
<i>Saline</i>					
HR					
NC	66±3*	4±1†	5±1†	7±2†	5±2†
HF	88±4	4±3†	6±2†	6±2†	7±2†
MBP					
NC	81±2	4±2†	3±1†	5±2†	3±2†
HF	81±4	3±1†	3±1†	3±1†	4±1†
<i>Acetylcholine</i>					
HR					
NC	65±2*	6±3†	6±2†	7±2†	6±2†
HF	89±3	2±1†	4±1†	3±2†	4±2†
MBP					
NC	84±1	2±2†	1±2†	1±1†	1±1†
HF	82±3†	4±1†	2±1†	1±1†	3±1†
<i>Saline</i>					
HR					
NC	68±2	10±2*†	9±2*†	9±2*†	10±2*†
HF	74±5	2±1	2±1	2±1	3±1
MBP					
NC	71±2	7±1†	10±2*†	11±2*†	11±2*†
HF	66±4	2±1	3±1	3±1	4±1
<i>Phentolamine</i>					
HR					
NC	65±3	6±1†	7±1†	9±1*†	10±1*†
HF	74±5	2±1	4±3	2±1	3±1
MBP					
NC	73±2*	5±1	8±3†	9±3†	10±3*†
HF	63±4	2±1	3±1	3±1	3±1
<i>Phentolamine + acetylcholine</i>					
HR					
NC	69±4	8±1*†	6±1†	6±1†	7±1*†
HF	78±9	2±1	3±1	4±1	4±1
MBP					
NC	71±3	5±2	8±2†	9±3†	9±3†
HF	64±5	2±1	3±1	3±2	3±2

Values are means ± SE. *Between-group difference (P < 0.05); †within-group difference (P < 0.05).

oxide stimulation, the increase in heart rate and mean blood pressure with saline infusion was similar between heart failure patients and normal controls. However, during protocol 2, under α₁-adrenergic blockade and cholinergic/nitric oxide stimulation, the increase in heart rate and mean blood pressure with saline infusion was more pronounced in normal controls.

Forearm blood flow during mental stress increased significantly in normal controls but not in heart failure patients, in whom forearm blood flow responses were significantly smaller (Fig. 2A). During mental stress, forearm vascular conductance increased significantly in normal controls (Fig. 2B). In contrast, in heart failure patients, forearm vascular conductance responses were essentially absent (Fig. 2B).

Hemodynamic Responses to Acetylcholine and Phentolamine at Rest

Resting heart rate and mean blood pressure in both normal controls and heart failure patients were not significantly changed by acetylcholine, phentolamine, and phentolamine associated with acetylcholine (Table 2).

Phentolamine infusion to eliminate the α-adrenergically mediated vasoconstriction significantly increased resting forearm blood flow in both normal controls and heart failure patients, although the levels of forearm blood flow remained signifi-

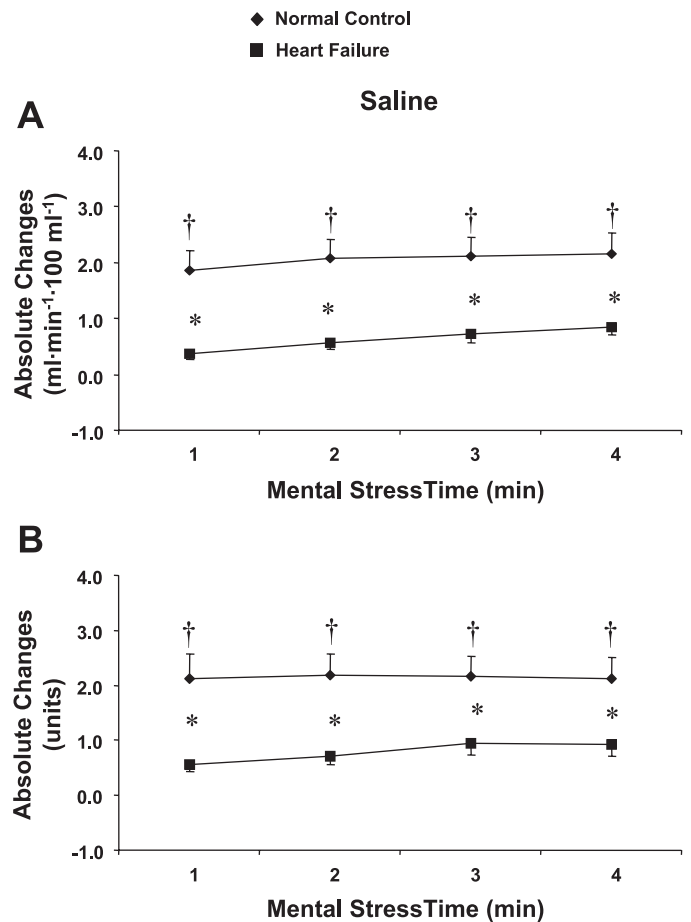


Fig. 2. FBF (A) and forearm vascular conductance (FVC; B) responses during mental stress with intra-arterial infusion of saline in normal controls and heart failure patients. Note that the vasodilatation is blunted in heart failure patients. †Within-group difference (P < 0.05); *between-group difference (P < 0.05).

cantly lower in heart failure patients (Fig. 3A). Phentolamine significantly increased resting forearm vascular conductance in heart failure patients and normal controls, and the differences between groups were no longer observed (Fig. 3B). Phentolamine associated with acetylcholine infusion to eliminate α -adrennergically mediated vasoconstriction and to stimulate cholinergically mediated nitric oxide production significantly increased resting forearm blood flow and forearm vascular conductance in normal controls and heart failure patients (Fig. 3, A and B). In addition, the significant differences between heart failure patients and normal controls were no longer observed.

Acetylcholine infusion to stimulate cholinergically mediated nitric oxide production significantly increased resting forearm blood flow and forearm vascular conductance in normal controls (Fig. 3, A and B). In heart failure patients, acetylcholine caused no significant change in resting forearm blood flow and forearm vascular conductance (Fig. 3, A and B). Thus resting forearm blood flow and forearm vascular conductance levels remained significantly lower in heart failure patients (Fig. 3, A and B).

Hemodynamic Responses to Mental Stress During Acetylcholine and Phentolamine

During mental stress with drug infusion, no significant difference in the levels of exertion perception during mental stress in heart failure patients and normal controls were found (data not shown).

Heart rate during mental stress with acetylcholine, phentolamine, and phentolamine associated with acetylcholine increased in both normal controls and heart failure patients. However, the increase in heart rate tended to be greater in normal controls than in heart failure patients (Table 2). Similarly, mean blood pressure during mental stress with acetylcholine, phentolamine, and phentolamine associated with acetylcholine increased in both normal controls and heart failure patients. The increase in mean blood pressure tended to be greater in normal controls (Table 2).

During mental stress in heart failure patients, acetylcholine administration caused no significant changes in both forearm blood flow and forearm vascular conductance (Fig. 4, A and B). When heart failure patients were compared with normal controls, forearm blood flow and forearm vascular conductance responses remained blunted during mental stress (Fig. 5, A and B).

In heart failure patients during mental stress, phentolamine significantly increased forearm blood flow (Fig. 4A). Similarly, phentolamine significantly increased forearm vascular conductance (Fig. 4B). When heart failure patients were compared with normal controls during mental stress, forearm blood flow remained significantly less during phentolamine administration (Fig. 5A). In regard to forearm vascular conductance, phentolamine administration tended to increase forearm vascular conductance to the normal levels (Fig. 5B). In heart failure patients during mental stress, phentolamine associated with acetylcholine provoked a marked increase in forearm blood flow (Fig.

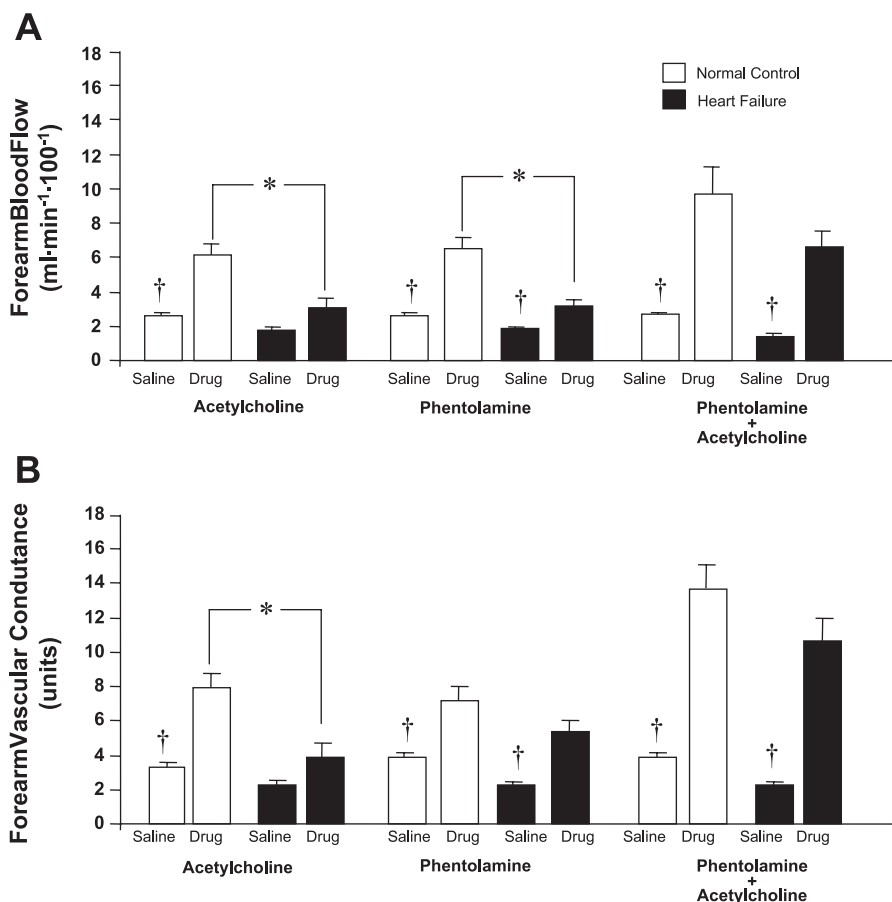


Fig. 3. Baseline FBF (A) and FVC (B) with intra-arterial infusion of saline, acetylcholine, phentolamine, and phentolamine plus acetylcholine in normal controls and heart failure patients. Note that no significant differences between groups were found after intra-arterial infusion of phentolamine plus acetylcholine. †Within-group difference ($P < 0.05$); *between-group difference ($P < 0.05$).

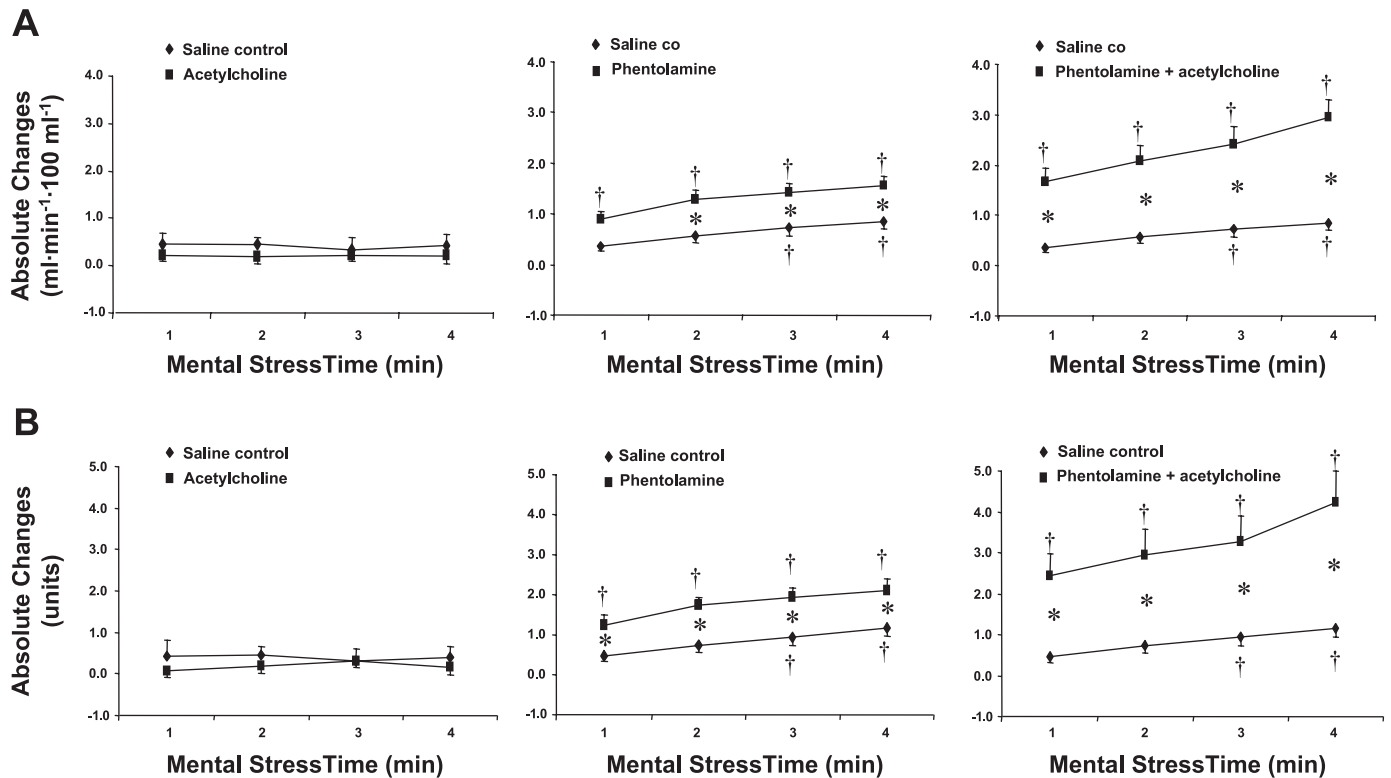


Fig. 4. FBF (A) and FVC (B) responses during mental stress with intra-arterial infusion of acetylcholine, phentolamine, and phentolamine plus acetylcholine in heart failure patients. Note that phentolamine and phentolamine plus acetylcholine significantly increased vasodilatation in heart failure patients. †Within-group difference ($P < 0.05$); *between-group difference ($P < 0.05$).

4A). Similarly, phentolamine associated with acetylcholine significantly increased forearm vascular conductance response during mental stress (Fig. 4B). When heart failure patients were compared with normal controls during mental stress, forearm blood flow increased toward the normal levels (Fig. 5A). Moreover, forearm vascular conductance responses were no longer different between groups (Fig. 5B).

DISCUSSION

In the present study, we observed that cholinergic/nitric oxide stimulation did not significantly increase forearm blood flow at rest or during mental stress in heart failure patients, but α_1 -adrenergic blockade alone, or associated with cholinergic/nitric oxide stimulation, improves forearm blood flow at rest and during mental in heart failure patients. These findings lead us to the conclusion that the exaggerated sympathetic nerve activity restrains endothelium-mediated reflex vasodilatation during mental challenge in chronic heart failure patients.

The muscle vasodilatation in response to mental stress depends on a competition between vasodilatory forces and vasoconstrictor forces. Thus the blunted forearm vasodilatation during mental stress in heart failure patients could represent attenuated vasodilatory factors, augmented vasoconstrictive forces, or both. In our prior work, we (10) reported that the blunted vasodilatation during mental stress in heart failure patients was not restored after an intra-arterial infusion of acetylcholine and L-arginine, thus confirming that abnormal vasodilatation is not the whole story. Interestingly, we (9) observed that the absolute muscle sympathetic nerve activity levels during mental stress were markedly elevated in heart

failure. The findings in the present study demonstrate that inhibition of α -adrenergic vasoconstrictor sympathetic nerve activity during mental stress largely restores forearm blood flow to normal, confirming that the excessive sympathetic nerve activity is the principal mediator of the blunted vasodilatory response during mental stress in patients with heart failure. Moreover, they provide evidence for a disequilibrium in favor of vasoconstrictor forces during mental stress in heart failure patients. However, it seems that the blunted vasodilatation in heart failure is a complex matter. Thus we cannot rule out the possibility that other vasoconstrictor mediators are implicated in the blunted vasodilatation in heart failure patients. A previous study (8) has demonstrated that after cyclooxygenase inhibition with indomethacin, the muscle vasodilatation was unchanged in normal controls but significantly increased in patients with heart failure. In addition, vitamin C improved the vasodilatation in response to cholinergic stimulation by acetylcholine in hypertensive and elderly patients (14). These findings suggest that prostanoids and oxidative stress, in addition to sympathetic overactivation, contribute to the blunted muscle vasodilatation during mental stress in chronic heart failure.

Although our study provides clear evidence that sympathetic vasoconstriction restrains muscle endothelium-mediated vasodilatation during mental stress in heart failure patients, it does not discriminate whether this inhibition affects the release of nitric oxide, prostacyclin, or endothelium-derived hyperpolarizing factor (5). However, there are two reasons favoring nitric oxide and endothelium-derived hyperpolarizing factor production. First, these two vasoactive autacoids are greatly enhanced

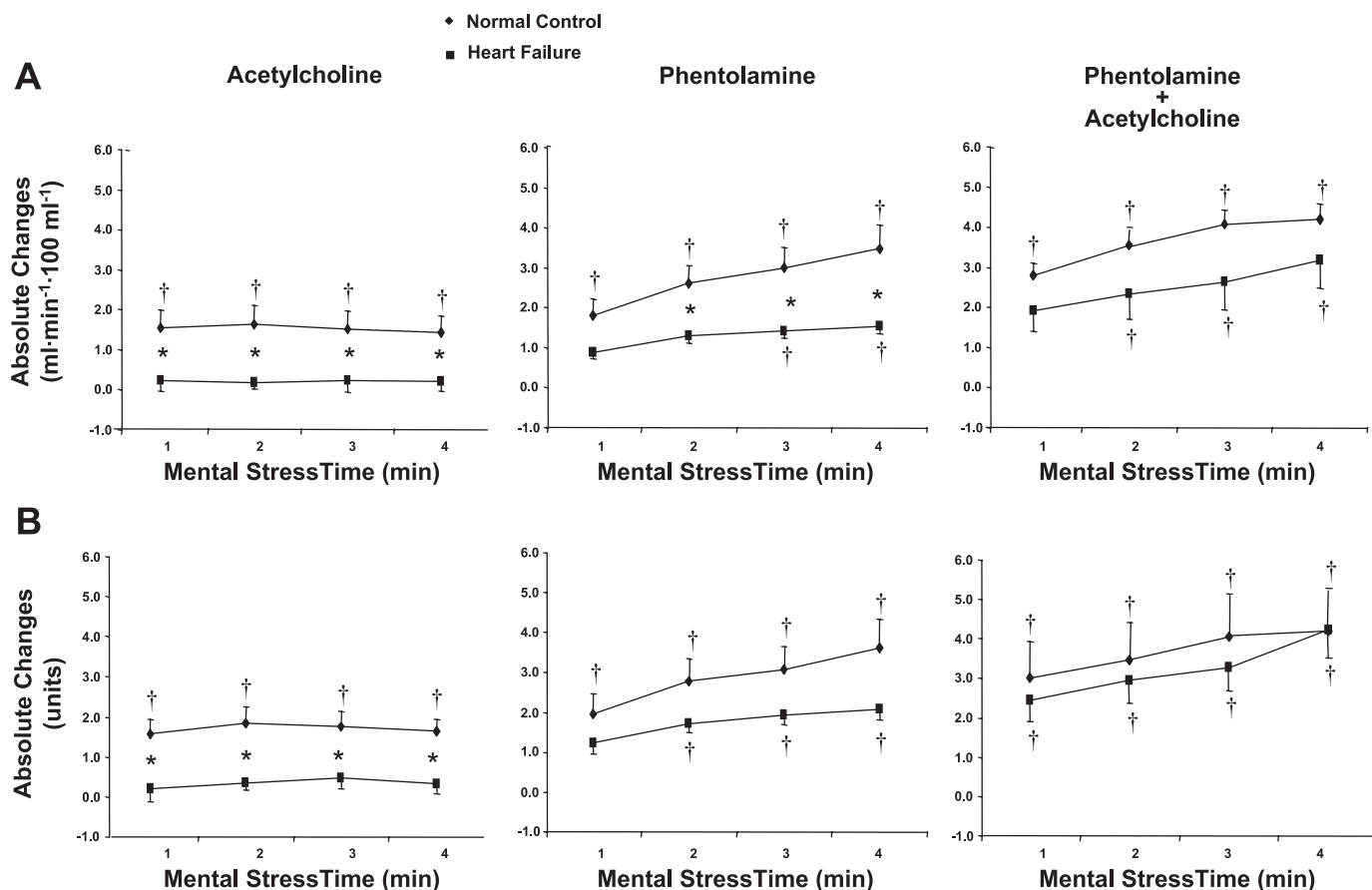


Fig. 5. FBF (A) and FVC (B) responses during mental stress with intra-arterial infusion of acetylcholine, phentolamine, and phentolamine plus acetylcholine in normal control and heart failure patients. Note that after phentolamine plus acetylcholine infusion, the significant differences between groups were no longer observed. †Within-group difference ($P < 0.05$); *between-group difference ($P < 0.05$).

in the presence of receptor-dependent acetylcholine stimulation (5). Second, the reflex muscle vasodilatation in heart failure was restored when acetylcholine was associated with α -adrenergic inhibition. This is a promising area for future investigation.

The intra-arterial infusion of vasoactive medications caused no significant changes in heart rate and mean blood pressure. These findings are suggestive that there was no systemic spillover of those medications. Thus our observations were specific for muscle vascular response independently of systemic circulation.

Limitations

We recognize limitations in this study. The blockade or stimulation of some blood vessel regulators can reflexively activate other mechanisms during mental stress. A previous study (1) showed that sympathectomy significantly reduced muscular blood flow during physiological maneuvers in humans. Thus we do not know whether the sympathetic blockade and cholinergic stimulation had other blood vessel implications that were not controlled in the present study.

The defense reaction that in humans can be elicited by mental stress depends on the levels of exertion perception, which can vary considerably among individuals. Thus the reduced vasodilatory response could be explained by a lower exertion during mental stress in heart failure patients. This is

unlikely because there were no significant differences in the levels of exertion perception throughout experimental protocols between heart failure patients and normal controls. In addition, the fact that heart rate and blood pressure continued to increase during each repetition of the mental stress is further evidence that it was still stressful, despite repeated sessions.

The individuals were not the same across all experimental protocols. Although we recognize that the same individuals across all experimental protocols could improve our study, on the other hand, the residual effects of medications and the reduction of exertion perception after many mental stress episodes could confound our interpretation.

The patients involved in this study were in advanced stage of heart failure. Therefore, we do not know whether sympathetic nerve activation would play a role in the vasodilatation during mental stress in moderate heart failure patients. This would be an interesting topic for future investigations. In addition, the medications were discontinued for 12 h before the study. This short period may be insufficient for medication washout. We recognize this limitation, but our patients were very sick and could not have their medications withdrawn for a longer period.

Perspectives

The present findings suggest that future strategies for treatment of heart failure based on pharmacological and nonphar-

macological therapies should take into consideration the sympathetic nerve activity. In fact, in recent study, we (12) found that an exercise training regimen for 4 mo dramatically reduced muscle sympathetic nerve activity in patients with chronic heart failure. Moreover, this sympathetic withdrawal was associated with a significant increase in forearm blood flow. Therefore, it is conceivable that exercise training improves muscle endothelium-mediated vasodilatation during mental stress in chronic heart failure.

GRANTS

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo Grant 01/0009-0 and in part by Fundação Zerbini. A. C. Santos was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo Grant 01/00615-8.

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