Limb Vascular Responsiveness to β-Adrenergic Receptor Stimulation in Patients With Congestive Heart Failure

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Background. In patients with congestive heart failure, the chronotropic and inotropic responses to β-adrenergic agonists are reduced. It is not known whether desensitization of peripheral β-adrenoceptors accounts for impaired limb vasodilation in these patients. Accordingly, we studied 14 normal subjects and 13 age-matched patients with congestive heart failure.

Methods and Results. To distinguish vasodilation mediated by β-adrenoceptors and adenylyl cyclase from that mediated by stimulation of guanylate cyclase, each subject received intra-brachial artery infusions of isoproterenol (1–100 ng/min) and sodium nitroprusside (0.3–10 μg/min), respectively. Forearm blood flow was determined by venous occlusion plethysmography. Maximal vasodilative potential, determined during reactive hyperemia, was reduced in the patients with congestive heart failure. The maximal forearm blood flow response to isoproterenol was comparable in patients with heart failure and in normal subjects (8.0±1.1 versus 9.2±1.2 ml/100 ml of tissue/min, respectively, p=NS). Furthermore, the dose–response relation to isoproterenol was similar in both groups. Likewise, the forearm vasodilative response to sodium nitroprusside was preserved in the heart failure group. Plasma concentration of norepinephrine was higher in the patients with heart failure (436±34 versus 201±74 pg/ml, p<0.01). When both groups were considered, there was no correlation between norepinephrine levels and the maximal forearm blood flow response to isoproterenol (r=0.10, p=NS).

Conclusions. We conclude that β-adrenoceptor desensitization does not occur in the limb vessels of patients with congestive heart failure. (Circulation 1991;83:1873–1879)

In experimental animals and patients with congestive heart failure (CHF), the chronotropic and inotropic responses to β-adrenergic receptor agonists are reduced.1–4 Increased sympathetic nervous system activity has been suggested to reduce β-adrenoceptor number or sensitivity.5,6 This, however, may have more of an effect on the β2-adrenoceptors that mediate myocardial function than on the β2-adrenoceptors that regulate limb blood flow.7–9 Studies of failing human left ventricles have demonstrated downregulation of β2- but not β1-adrenoceptors.10 Furthermore, the contractile responses to selective β1-adrenoceptor agonists are reduced, whereas those to β2-adrenoceptor agonists are preserved.10 Nonetheless, one cannot necessarily extrapolate findings derived from the myocardium of patients with CHF to other tissues, even though cardiac β2-adrenoceptor number is preserved and lymphocyte β2-adrenoceptor density is decreased.11

Accordingly, the purpose of this study was to determine whether the limb vascular response to β-adrenoceptor stimulation is impaired in patients with CHF. This information has clinical relevance because it would provide insight as to why limb blood flow is reduced during exercise in these patients. If β2-adrenoceptor function is abnormal in the vessels supplying the skeletal muscles of these individuals, the vasodilative response to increasing catecholamine concentrations during exercise would be blunted, and blood flow augmentation would be attenuated.

Methods

Subjects

The control subject population in this study was made up of 14 normal volunteers, consisting of 12 men and two women, whose ages ranged from 26 to

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Experimental women potential 14% or and smooth muscle This prusside. received Brigham and heart disease in three patients, and primary cardiomyopathy in six patients. Two patients were in New York Heart Association functional class I, two were in class II, eight were in class III, and one was in class IV. Left ventricular ejection fraction determined by angiography or radionuclide ventriculography ranged from 14% to 32% (average, 22±6%). Digoxin, diuretics, and all vasoactive medications were withheld at least 24 hours before intervention. This study was approved by the human research committee at Brigham and Women’s Hospital, and each subject gave written, informed consent.

Experimental Protocol

Each participant was studied in the postabsorptive state in a 22°C temperature-controlled room. Alcohol, caffeine, and cigarettes were prohibited within 12 hours of the study. Under local anesthesia and sterile conditions, a polyethylene catheter was inserted into a brachial artery of each study participant for determination of blood pressure and for infusion of drugs. The Vascular Research Laboratory was quiet, and lights were dimmed. All study participants rested at least 30 minutes after catheter placement to establish a stable baseline before data collection.

During the control period, measurements of forearm blood flow and blood pressure were repeated every 10 minutes until stable. A solution of 5% dextrose was infused intra-arterially at a rate of 0.4 ml/min during this period. To determine the maximal vasodilative potential of the resistance vessels, forearm blood flow was measured in the basal state and during reactive hyperemia after 5 minutes of an ischemic stimulus. Ischemia was induced by inflation of a sphygmomanometric cuff on the upper arm to suprasystolic pressure. Forearm blood flow measurements were repeated until basal conditions were reestablished.

To evaluate β-adrenoceptor responsiveness, isoproterenol was infused into the brachial artery. Forearm blood flow was measured during infusion of increasing concentrations of isoproterenol at dosages of 1, 3, 10, 30, and 100 ng/min, each for 5 minutes, delivered at a rate of 0.4 ml/min. Thereafter, 5% dextrose was again infused, and baseline conditions were reestablished. To further evaluate vascular smooth muscle relaxation, each study participant received an intra-arterial infusion of sodium nitroprusside. This agent, which acts directly on vascular smooth muscle to stimulate soluble guanylate cyclase, was given at dosages of 0.03, 0.3, 3, and 10 μg/min, at a rate of 0.4 ml/min, each for 5 minutes. Thus, one could distinguish vasodilation mediated by β-adrenoceptors and adenylate cyclase from that mediated by an agonist that induces vasodilation by activation of guanylate cyclase.

Hemodynamic Measurements

Bilateral forearm blood flow was determined by venous occlusion strain-gauge plethysmography, by use of calibrated mercury-in-Silastic strain gauges, and as expressed as milliliters per 100 milliliters of tissue per minute (D.E. Hokansen, Inc., Issaquah, Wash.). Each arm was supported above heart level. Venous occlusion pressure averaged 35±5 mm Hg. Circulation to the hand was arrested by inflating a wrist cuff to suprasystolic pressures before each measurement of forearm blood flow. Each forearm blood flow determination comprised at least five separate measurements performed at 10–15-second intervals. By measuring blood flow in the infused arm, one can determine the direct effect of the vasoactive drug. By measuring blood flow in the noninfused arm, one can be assured that systemic effects have not occurred if no changes in blood flow developed during the drug infusion. Forearm vascular resistance was calculated as the ratio of mean blood pressure to forearm blood flow and expressed as units, which reflected millimeters of mercury per milliliter per 100 milliliters of tissue per minute.

Blood pressure was measured by an arterial cannula that was attached to a Statham P23 pressure transducer (Gould-Statham, Oxnard, Calif.), aligned to an amplifier on a Gould physiologic recorder (Gould-Statham). Heart rate was determined from a simultaneously obtained electrocardiographic signal and calculated from the RR interval.

Blood samples for determination of plasma concentrations of norepinephrine and epinephrine were withdrawn after study participants had rested in the supine position for at least 30 minutes after cannula placement. Samples were placed immediately on ice when collected and were centrifuged at 2°C. Plasma samples were stored at −70°C before assays. Plasma concentrations of norepinephrine and epinephrine were quantified by a modified radioenzymatic assay.

Statistical Analysis

Forearm blood flow, blood pressure, and heart rate data are presented as mean±SEM. All other data are presented as mean±SD. Statistical analysis was performed with analysis of variance of independent groups for repeated measures, followed by Newman-Keuls post hoc testing for statistical significance. For nonserial measurements, the Student’s t test was used to analyze the difference between the means within each group. The unpaired t test was used to compare the basal conditions between each group. Linear regression analysis was performed for selected hemodynamic variables. Statistical significance was accepted at the 95% confidence level (p≤0.05).
Results

Basal and Reactive Hyperemic Forearm Blood Flow

Basal forearm blood flow in normal subjects and CHF patients were comparable: 2.7±0.2 and 2.7±0.5 ml/100 ml of tissue/min, respectively (p=NS) (Figure 1). Similarly, forearm vascular resistance at baseline was 36.1±4.3 units in normal subjects and 41.3±4.9 units in CHF patients (p=NS). Reactive hyperemic blood flow, however, was different between the two groups. In normal subjects, it was 23.5±1.5 ml/100 ml of tissue/min; in CHF patients, it was significantly less and was 18.7±2.2 ml/100 ml of tissue/min (p<0.05). Minimal vascular resistance determined during reactive hyperemia was 3.6±0.3 and 5.5±0.7 in normal subjects and in CHF patients, respectively (p<0.05).

Response to Isoproterenol

Mean blood pressure at baseline was 83±4 mm Hg in normal subjects and 89±4 mm Hg in CHF patients (p=NS). Basal heart rate was 61±2 and 78±4 beats/min in normal subjects and in CHF patients, respectively (p<0.01). The incremental intra-arterial infusion of isoproterenol caused no changes in systemic blood pressure or heart rate in either group.

The forearm blood flow response to isoproterenol in normal subjects and in CHF patients is illustrated in Figure 2. The maximal forearm blood flow response to isoproterenol in normal subjects was 9.2±1.2 ml/100 ml of tissue/min and in CHF patients was 8.0±1.1 ml/100 ml of tissue/min (p=NS). Furthermore, the dose–response curve to isoproterenol was similar in both groups. No changes in forearm blood flow occurred in the noninfused arm in either group. Because there was no change in blood pressure, the change in forearm blood flow during the isoproterenol infusion reflected changes in forearm vascular resistance. Forearm vascular resistance was similar in normal subjects and in CHF patients at all doses of isoproterenol.

To exclude confounding effects caused by differences in postreceptor vascular smooth muscle function, the forearm blood flow response to isoproterenol was analyzed as a proportion of the reactive hyperemic blood flow in each normal subject and CHF patient. Again, there was no significant difference in the dose–response relation to isoproterenol between the two groups. These data indicate that the limb vasodilative responsiveness to β-adrenoceptor stimulation is not reduced in CHF patients.

Response to Sodium Nitroprusside

To determine whether CHF affects the ability of the vascular smooth muscle to dilate in response to other agonists, the vasodilative response to sodium nitroprusside was examined. Intra-arterial infusion of sodium nitroprusside did not change mean blood pressure or heart rate in either group. The maximal forearm blood flow response to sodium nitroprusside in CHF patients was similar to that observed in normal subjects (10.5±1.1 and 11.3±1.2 ml/100 ml of tissue/min, respectively, p=NS) (Figure 3). Furthermore, there was no difference between CHF patients and normal subjects in the dose–response curve to sodium nitroprusside. Likewise, changes in forearm vascular resistance induced by sodium nitroprusside were similar in each group. No change in forearm blood flow or forearm vascular resistance occurred in the noninfused arm in either group.

Relation of Plasma Levels of Catecholamines to β-Adrenoceptor Responsiveness

A rationale for the hypothesis tested in this study was that increased sympathetic nervous system activity, as reflected in high circulating levels of norepinephrine, would depress β-adrenoceptor sensitivity in CHF patients. Indeed, plasma concentration of norepinephrine in CHF patients was significantly higher than that detected in normal subjects (436±34 versus 201±74 pg/ml, respectively, p<0.01). Plasma concentration of epinephrine was 78±18 and 57±11...
pg/ml in CHF patients and normal subjects, respectively (p=NS).

Linear regression analysis was performed to determine whether the limb vasodilative response to isoproterenol was impaired only in those patients with the highest levels of plasma norepinephrine or epinephrine. When all participants were included in the analysis, no correlation existed between basal plasma concentration of norepinephrine and the maximal forearm blood flow response to isoproterenol (r = -0.10, p = NS) (Figure 4, upper panel). Likewise, there was no correlation between basal plasma concentration of epinephrine and the maximal forearm blood flow response to isoproterenol (r = -0.02, p = NS) (Figure 4, lower panel). When only the CHF patients were included in the analysis, however, there was a significant negative correlation between plasma concentrations of norepinephrine (r = -0.57, p < 0.05) as well as epinephrine (r = -0.54, p < 0.05) and the maximal forearm blood flow response to isoproterenol. This observation, however, does not imply necessarily that elevated concentrations of catecholamines reduce β-adrenoceptor responsiveness because those patients with the highest plasma levels of norepinephrine and epinephrine tended to have more severe CHF and less ability to vasodilate with any stimulus. Indeed, in the patients with heart failure, both plasma concentrations of norepinephrine and epinephrine correlated inversely with the maximal blood flow response to nitroprusside (r = -0.58, p < 0.05 and r = -0.73, p < 0.01, respectively) and also with reactive hyperemic blood flow (r = -0.55, p < 0.05 and r = -0.56, p < 0.05, respectively).

Discussion

It is now well established that β-adrenoceptor function is abnormal in the myocardium of patients with CHF. In failing ventricles, there are decreased numbers of β-adrenoceptors, reduced isoproterenol-stimulated adenylate cyclase activity, and depressed inotropic and chronotropic responsiveness to β-adrenoceptor agonists.1-4,10 This investigation is the first to examine β-adrenoceptor function in the limbs of humans with CHF.

Limb Vasodilative Response to Isoproterenol

We found no impairment in isoproterenol-mediated limb vasodilation in CHF patients compared with a normal control population. It must be emphasized that our two groups were age matched because β-mediated responsiveness diminishes with age.15-17 Our findings in humans are comparable to those observed in a canine model of CHF recently reported by Frey et al.18 In that study, isoproterenol-induced hind limb vasodilation was similar in control dogs and dogs with CHF.

The fact that β-adrenoceptor regulation of limb blood flow is preserved but that β-adrenergic effects on heart rate and contractility are reduced may reflect preferential downregulation of β1-receptors by neurally released and circulating norepinephrine. Bristow et al.19 studied β1- and β2-adrenoceptors in failing human ventricles.10 They examined isolated cardiac tissue using radioligand-binding techniques.
and reported that β₁-adrenoceptor downregulation occurred in the failing left ventricle, whereas there was no change in β₂-adrenoceptors. Furthermore, there was a decreased contractile response of isolated trabeculae to β₁- but not to β₂-stimulation. It should be acknowledged that in vivo dose–response curves reflecting forearm blood flow responses to isoproterenol may not detect small abnormalities in β₂-receptor pathway function. Indeed, the contractile response of failing ventricles to β₂-adrenoceptor stimulation is preserved, even though these receptors are partially uncoupled from adenyl cyclase.19

It has been suggested that reduced β₂-adrenoceptor responsiveness in CHF occurs as a consequence of increased sympathetic nervous system activity and neural release of norepinephrine.20,21 Cultivation of rat myocardial cells in the presence of norepinephrine downregulates β₂-adrenoceptors and reduces isoproterenol and forskolin-stimulated AMP formation.22 Moreover, incubation of other cell lines such as C6 rat glioma cells with norepinephrine or S49 mouse lymphoma cells and human fibroblasts with isoproterenol desensitizes β₂-adrenoceptors.23-26 In some experimental models, increased circulating levels of norepinephrine uncouple the β₂-adrenoceptor from its second messengers but does not reduce receptor density.6,27 Conversely, in patients with CHF, β₂-adrenoceptor blockade increases receptor number and function.28

The fact that norepinephrine has greater β₂- than β₂-adrenergic effects may explain the selective downregulation of β₂-adrenoceptors in patients with CHF.29 Intravenous administration of norepinephrine for 10 days to rabbits decreased β₂-adrenoceptor density in the heart but not in lymphocytes, which are blood cells that possess predominantly β₂-receptors.30 In contrast, infusion of clenbuterol, a β₂-adrenoceptor agonist, to rats reduced skeletal muscle β₂-adrenoceptor (presumably β₁) density.31 In normal humans, administration of procaterol, a β₂-adrenoceptor agonist, decreased lymphocyte β₂-adrenoceptor density; xamoterol, a β₂-adrenoceptor agonist, decreased β₂-receptor function but did not affect β₂-adrenoceptor function.32 In patients with CHF, the β₂-adrenoceptor agonist, pirbuterol, reduced lymphocyte β₂-receptor density.11 Indeed, plasma concentration of norepinephrine was elevated in our patients with CHF, whereas the concentration of epinephrine, a hormone with more balanced β₁- and β₂-adrenoceptor properties, was similar in each group.

**Rationale for Examining Peripheral β-Receptors**

The limb blood flow response to exercise is reduced in patients with CHF.33-36 Several mechanisms have been proposed to account for this abnormality and include competing vasoconstrictive stimuli secondary to activation of the sympathetic nervous system and the renin-angiotensin system, reduced perfusion pressure resulting from left ventricular systolic dysfunction, and impaired vasodilative capacity secondary to increased stiffness in the resistance vessels. Zelis and colleagues37 and Sinoway et al38 reported that the dilating capacity of forearm resistance vessels during both reactive hyperemia and hand exercise was reduced in patients with decompensated CHF. Wilson and colleagues,39 however, studied nonedematous patients with CHF and found no reduction in the vasodilative response to reactive hyperemia or arm exercise. In our investigation, reactive hyperemic blood flow was decreased in the CHF group, including those individuals who did not have peripheral edema.

One mechanism that had not been investigated in humans, however, was whether β₂-adrenoceptor–mediated vasodilation of skeletal muscle was reduced in patients with CHF. These receptors are activated by exogenously administered agonists or by circulating catecholamines when concentrations of the latter are increased during exercise. Therefore, if skeletal muscle β₂-receptors contribute to vasodilation during exercise, and some investigators suggest that they do not, reduced receptor function could account for exercise intolerance in CHF.40 This study does not support that hypothesis because the forearm blood flow response to isoproterenol was preserved in the patients with CHF. It is conceivable, albeit unlikely, that β₂-adrenoceptor responsiveness would be reduced in an exercising extremity.41,42

**Limb Vasodilative Response to Nitroprusside**

Prior studies examining vasodilator function in patients with heart failure have yielded different conclusions. In the aforementioned report by Zelis et al37 involving CHF patients with peripheral edema, the vasodilative responses to sodium nitrite and phenolamine were attenuated compared with normal subjects. Kubo et al,43 however, reported that the vasodilative responses to phenolamine and yohimbine were preserved in patients with CHF. If an abnormal response to isoproterenol had been detected, we wanted to be certain that this did not reflect a general depression of vascular smooth muscle responsiveness in our CHF patients. For this reason, we measured forearm blood flow during an intra-arterial infusion of sodium nitroprusside, a smooth muscle relaxant that acts by the guanylate cyclase pathway. As was observed with isoproterenol, the forearm vascular response to sodium nitroprusside was similar in both groups.

Exception to these observations can be made in those few patients with the most severe manifestations of CHF. In these patients, generalized impairment occurred during vasodilation, which was reflected in the reduced blood flow responses to reactive hyperemia, nitroprusside, and isoproterenol. Even though plasma norepinephrine concentrations were highest in these individuals, one cannot cite β₂-adrenoceptor downregulation as a cause of the vasodilative dysfunction because catecholamine levels also correlated inversely with reactive hyperemic blood flow and the maximal blood flow responses to
nitroprusside. Perhaps a study comprising only patients with end-stage CHF would demonstrate generalized impairment of vasodilation. We believe that the patients enrolled in the study, however, are representative of most patients with CHF.

Conclusions
In these patients with CHF, the forearm blood flow response to reactive hyperemia was impaired, suggesting that structural changes occur in the resistance vessels that limit maximal vasodilation. However, the vasodilator effects of incremental doses of isoproterenol, as well as sodium nitroprusside, were similar to those observed in age-matched normal subjects. We conclude that limb β-adrenoceptor responsiveness is preserved in patients with CHF.

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References

KEY WORDS • congestive heart failure • isoproterenol • blood flow, forearm • β-adrenergic receptor • epinephrine • norepinephrine
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