Intracoronary infusion of dobutamine to patients with and without severe congestive heart failure. Dose-response relationships, correlation with circulating catecholamines, and effect of phosphodiesterase inhibition.

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Research Article

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Intracoronary Infusion of Dobutamine to Patients with and without Severe Congestive Heart Failure

Dose-response Relationships, Correlation with Circulating Catecholamines, and Effect of Phosphodiesterase Inhibition

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Abstract

We infused dobutamine into the left main coronary artery of 24 patients with severe congestive heart failure (CHF) and 8 normal subjects without hemodynamic dysfunction. The maximal +d*P*/dt response to intracoronary (IC) dobutamine in CHF patients was only 37% of that in normals. This decrease in maximal response was not associated with a rightshift in the EC_{50} for dobutamine's effect on +d*P*/dt, or a decrease in the affinity of myocardial beta adrenergic receptors for dobutamine determined in vitro. In nine of the CHF patients, IC dobutamine infusion was followed by IC infusion of the phosphodiesterase inhibitor milrinone, and subsequently, by a second IC infusion of dobutamine. After IC milrinone, the increase in +d*P*/dt caused by IC dobutamine (74±10%) was significantly greater than that caused by the first infusion of dobutamine (52±11%; $P < 0.003$) or milrinone (42±6%; $P < 0.001$). Resting plasma norephinephrine was markedly elevated in CHF patients (837±208 ng/liter), but not in normal subjects (142±32 ng/liter); and the increase in +d*P*/dt caused by IC dobutamine was inversely related to resting plasma norepinephrine levels ($r = -0.653$; $P < 0.001$). IC dobutamine caused a dose-related decrease in plasma norepinephrine (maximal effect, −160±31 ng/liter; $P < 0.001$). Thus, (*a*) the maximal inotropic response to dobutamine is markedly depressed in patients with severe CHF, and is significantly greater after pretreatment with the phosphodiesterase inhibitor milrinone; (*b*) the impairment in inotropic response to dobutamine is inversely related to circulating norepinephrine levels; and (*c*) myocardial stimulation by dobutamine results in withdrawal of sympathetic tone.

Introduction

Stimulation of myocardial beta adrenergic receptors causes activation of adenylate cyclase, and consequently, increased accumulation of adenosine 3',5'-monophosphate (cAMP), which is thought to augment myocardial contractility by increasing the influx of calcium through voltage-dependent channels (1). In myocardium from patients with severe congestive heart failure (CHF),[1] Bristow et al. have shown that the density of beta adrenergic receptors is decreased, as are the activation of adenylate cyclase and the development of contractile force in response to isoproterenol or dobutamine (2–4). Nevertheless, there is evidence that myocardium from patients with severe CHF responds normally to calcium and cAMP (2–7). These important observations have implications regarding the pathophysiology and therapy of CHF, and raise several relevant questions. (*a*) What is the in vivo functional hemodynamic consequence of beta adrenergic desensitization in patients with CHF? (*b*) Can the hemodynamic sequelae of beta adrenergic desensitization be overcome at high levels of receptor stimulation? (*c*) Can the myocardial response to beta adrenergic stimulation be restored by inhibiting the degradation of cAMP? (*d*) Is there a relationship between the level of basal sympathetic nervous system activation, as reflected by plasma catecholamines, and the degree of beta adrenergic desensitization?

Recently, we found that the positive inotropic and hemodynamic effects caused by the myocardial actions of milrinone, a phosphodiesterase inhibitor, could be quantitated by direct intracoronary (IC) drug infusion (8). This approach allows isolation of the myocardial actions of the infused agent, the precise definition of dose-response relationships, frequently at drug concentrations greater than those that can be achieved during systemic infusion, and the use of left ventricular +d*P*/dt as a simple, quantifiable measure of the positive inotropic response to the infused drug. In a prior report (9), we described the differential positive inotropic responses to an IC infusion of the beta adrenergic agonist dobutamine (10) to patients with and without severe congestive heart failure. We now report the detailed dose-response relationships for the positive inotropic and hemodynamic effects of IC dobutamine infusion to 24 patients with severe CHF and 8 subjects without significant myocardial dysfunction; and the use of this methodology as an approach to the aforementioned questions.

Methods

Study population. The study population consisted of 32 subjects and was divided into two groups. One group ("normals," $n = 8$) had no evidence of significant CHF. These patients (three males, five females;

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1. *Abbreviations used in this paper:* CHF, congestive heart failure; D_5W, 5% dextrose in water; d*P*/dt, derivative of pressure with respect to time; IC, intracoronary; IPIN, iodopindolol; LV, left ventricular.

mean age±SEM, 52±2 yr) had normal baseline hemodynamic function at the time of study (Figs. 3 and 4). None had a history of congestive heart failure symptoms, exhibited cardiomegaly on chest x-ray, or had been treated for congestive heart failure. Seven were receiving no cardiac medications before the time of study, and one received a beta adrenergic blocking drug until the night before study for a chest pain syndrome. The other group ("CHF," $n = 24$) consisted of patients with idiopathic ($n = 8$) or ischemic ($n = 16$) dilated cardiomyopathy who were referred because of severe New York Heart Association functional class III or IV CHF despite treatment with digitalis, diuretics, and vasodilators in all cases. There were 20 males and 4 females (mean age, 59±2 yr). The mean left and right ventricular ejection fractions by radionuclide angiography were 0.15±0.02 and 0.31±0.03, respectively. All CHF patients received digitalis and diuretics up to and including the day before study, whereas vasodilators were withheld for at least 48 h before study. No subject had symptomatic coronary artery disease, documented myocardial infarction within the previous 3 mo or uncorrected valvular heart disease. The study protocol was approved by the Committee for the Protection of Human Subjects from Research Risks at the Brigham and Women's Hospital, and written informed consent was obtained in all cases.

Hemodynamic measurements. Under local anesthesia, the following catheters were placed: (*a*) a 7Fr triple-lumen Swan-Ganz catheter with a right atrial port was placed in the right or left main pulmonary artery; (*b*) an 8Fr micromanometer-tipped catheter (Millar Industries, Houston, TX) was placed in the left ventricle; (*c*) a 7Fr L-4 Judkins catheter was placed in the ostium of the left main coronary artery as would be done for routine contrast injection; and (*d*) a side-arm sheath (Cordis Laboratories, Miami, FL) in the right femoral artery was used to monitor arterial pressure. Left ventricular (LV) +dP/dt was measured and hemodynamic calculations were performed as previously described in detail (11).

Plasma catecholamines and dobutamine. Plasma norepinephrine, epinephrine, and dobutamine concentrations were determined in blood drawn from the femoral artery. All patients were supine and resting for at least 30 min after placement of catheters. Plasma catecholamines and dobutamine were determined by radioenzymatic assay (12). Dobutamine coseparates with dopamine. Since even the lowest pharmacologic concentrations of dobutamine (17–40 μg/liter) (reference 15 and see Fig. 1 *B*) are severalfold higher than the highest endogenous dopamine levels in our patients (range in this study, 0.021 to 0.831 μg/liter; mean = 0.132±0.059 μg/liter), the contribution of dopamine to the overall dobutamine determination is minimal. The dobutamine standard curve using this method was linear from 2.5 to 250 μg/liter.

Intracoronary dobutamine infusion. After placement of catheters, baseline hemodynamic conditions were taken as the means of two consecutive sets of hemodynamic measurements separated by at least 10 min and differing by < 10%. Subsequently, 5% dextrose in water (D_5W), the vehicle for IC drug infusion, was infused into the left main coronary artery for 5 min. At an infusion rate (4 ml/min) that exceeded the maximum infusion rate used for the subsequent drug infusions, D_5W had no effect on +dP/dt (Fig. 2) or any of the hemodynamic measurements. Dobutamine diluted in D_5W was then infused with upward titration of the infusion rate at 5-min intervals according to the following schedule: 2.5, 5, 12.5, 25, 50, 100, and 200 μg/min. Hemodynamic measurements were made during the fifth minute of each infusion period, and the upward titration was continued until the 200-μg/min infusion rate was achieved or there was evidence of an adverse effect such as excessive tachycardia, increased ventricular ectopic activity, or unpleasant palpitations.

Assuming a left main coronary artery blood flow of 125 ml/min (13), the approximate concentration of dobutamine in the coronary artery would range from 0.066 to 5.30 μM over infusion rates ranging from 2.5 to 200 μg/min. Peripheral arterial dobutamine concentration was measured in 11 patients during IC dobutamine infusion (Fig. 1 *a*), and compared to that in 17 previously reported patients with a similar degree of CHF (14) during intravenous dobutamine (Fig. 1 *B*). During intravenous dobutamine infusion, peripheral dobutamine concentrations ranged from 17±1 to 162±27 μg/liter at intravenous infusion rates of 2 to 14 μg/kg per min (Fig. 1 *B*), in good agreement with the previous report of Leier et al. (15). At an IC infusion rate of 25 μg/min, the estimated IC dobutamine concentration is 200 μg/liter, a level similar to or greater than that achieved during intravenous drug infusion at the maximum approved rate of 14 μg/kg per min (Fig. 1 *B*). At this IC infusion rate (i.e., 25 μg/min) peripheral dobutamine was barely detectable (6±2 μg/liter), and was substantially less ($P < 0.001$) than the concentration of drug achieved with a 2-μg/kg per min intravenous drug infusion (17±1 μg/liter). A plateau in the +dP/dt response generally occurred by the end of the second minute, and in all cases by the end of the fourth minute of IC dobutamine infusion; and was stable for the remainder of each 5-min infusion period. After discontinuing

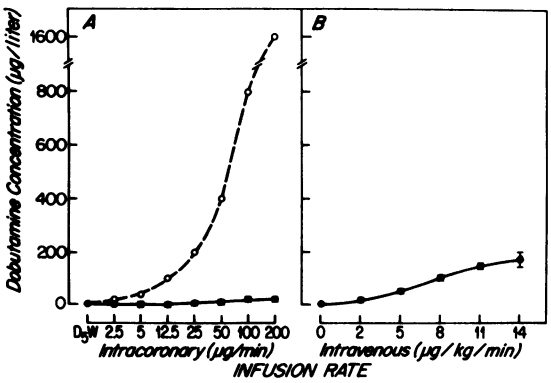

Figure 1. Relative peripheral and coronary artery concentrations of dobutamine during intravenous and IC infusion to patients with New York Heart Association functional class III and IV CHF. (*A*) Concentration of dobutamine measured in plasma (●) and estimated in the left main coronary artery (○) during IC infusion. The left main coronary artery concentration of dobutamine was calculated based on an assumed blood flow of 125 ml/min (13). (*B*) Measured plasma dobutamine concentration in 17 patients with New York Heart Association functional class III and IV CHF during intravenous drug infusion.

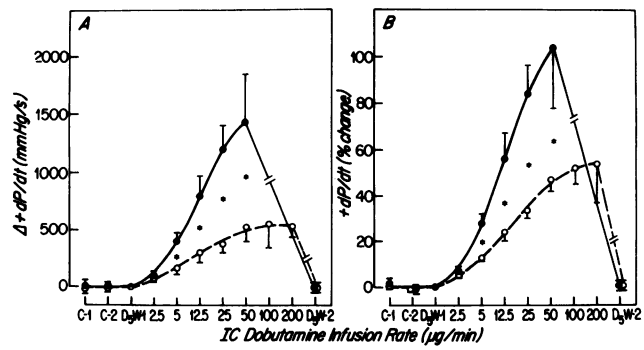

Figure 2. Dose-response relationship for the effects of IC dobutamine infusion on left ventricular +dP/dt in patients with (○) and without (●) CHF. (*A*) Absolute change in +dP/dt compared to the D_5W-1 baseline. Baseline +dP/dt was 1,446±87 and 886±97 mmHg/s in patients without and with CHF, respectively. The increase in +dP/dt over baseline was significant at all infusion rates in both groups. At 5 μg/min and all higher infusion rates, the increase was greater in patients without CHF. *$P < 0.01$ for normal vs. CHF. (*B*) Data from A presented as the percentage increase in +dP/dt over the D_5W-1 baseline. *$P < 0.01$ for normals vs. CHF.

IC dobutamine, +dP/dt and hemodynamics returned to baseline generally within 2 min, and in all cases within 5 min.

Intracoronary milrinone. In nine of the subjects with CHF, the recontrol period after dobutamine infusion was followed by the IC infusion of milrinone according to an upward titration at 5-min intervals as follows: 1.5, 3, 6, 12.5, 25, 50, 100, 200, and 400 μg/min. During IC milrinone infusion, hemodynamic measurements were made at the end of each 5-min infusion, and the upward titration was continued until +dP/dt increased by at least 30% over the D_5W baseline, the 400-μg/min infusion rate was reached, or there was evidence of an adverse effect such as excessive tachycardia or increased ventricular ectopic activity, as previously described in detail (8). In these nine subjects, the mean IC dobutamine infusion rate was 61±16 μg/min, and the mean IC milrinone infusion rate was 250±38 μg/min. Immediately after discontinuation of IC milrinone, IC dobutamine was infused for a second time at the same rate as before IC milrinone, and +dP/dt and hemodynamics were measured between 3.5 and 5.0 min later. In our previous study (8) we monitored +dP/dt after the discontinuation of IC milrinone infusion, and found that the +dP/dt response was maintained without significant decrease for at least 5, and generally 10–20 min, after discontinuing IC milrinone (unpublished observation). To control for the possibility that the first dobutamine infusion might have induced a degree of beta adrenergic receptor desensitization, IC dobutamine was infused for a second time after the initial IC dobutamine infusion in five other CHF subjects. In these patients, recontrol conditions were obtained after the initial IC dobutamine infusion, and subsequently, IC dobutamine was infused for a second time, at a rate equal to the last rate infused during the first dobutamine titration.

Receptor binding studies. Beta adrenergic receptor affinity for dobutamine was determined from competition binding curves for the [^{125}I]iodopindolol (IPIN) binding site essentially as described by Marsh and Smith (16). Cardiac membranes (average weight, ~ 500 mg) from left ventricle were obtained at the time of transplantation (CHF patients) or noncardiac organ donation (non-CHF patients), and were placed immediately in liquid N_2. Just before assay, specimens were warmed to 4°C, minced in 10 mM $NaHCO_3$, 10 mM histidine, pH 7.5; and homogenized by three bursts (10 s each) at full speed of a homogenizer (Polytron; Brinkmann Instruments, Westbury, NY). The homogenate was centrifuged at 40,000 g for 30 min, and the pellet was resuspended in assay buffer (10 mM Tris, 150 mM NaCl) by 20 strokes of a tight-fitting Dounce homogenizer. The membrane preparation (300 μl) was added to tubes containing IPIN (20–40 pM) and 18 graded concentrations of *dl*-dobutamine (10^{-4}–10^{-9} M), followed by incubation for 120 min at 37°C. The assay (performed in duplicate) was terminated by adding 3 ml of ice-cold buffer, followed by rapid filtration through a Whatman GF/B glass fiber filter presoaked in 0.5% aqueous polyethylenimine solution. Filters were washed thrice with ice-cold buffer, dried, and trapped radioactivity determined by scintillation spectrometry. Dobutamine competition curves were analyzed by computer-assisted nonlinear curve-fitting using a modification of the LIGFIT program (17) as previously described (16).

Statistical methods. All data are presented as the mean±standard error of the mean. Significant changes among multiple observations for each variable were detected by two-tailed paired t tests using Bonferroni's method for multiple simultaneous comparisons such that statistical significance was assumed if the null hypothesis could be rejected at the $0.05/n$ probability level (where n = the number of comparisons). Changes among two observations for one variable within the same group were determined by two-tailed paired t tests, and were considered significant if the null hypothesis could be rejected at the 0.05 probability level (18).

Results

Effect of IC dobutamine infusion on +dP/dt (Fig. 2). Baseline +dP/dt was 1,446±87 and 886±97 mmHg/s in normals and CHF patients, respectively ($P < 0.001$; normal vs. CHF). In both normal individuals and CHF patients, +dP/dt was significantly increased by IC dobutamine infusion at 2.5 μg/min, the lowest infusion rate studied. In normals, IC dobutamine infusion rates up to 50 μg/min resulted in further increases in +dP/dt, the peak increase for the group averaging 1,437±404 mmHg/s (+104±14%). In CHF patients, the IC dobutamine infusion was continued to a maximum infusion rate of 200 μg/min, and caused a 528±73 mmHg/s (+56±6%) increase in +dP/dt over baseline. At every infusion rate > 2.5 μg/min, the increase in +dP/dt was significantly greater in normals than in CHF patients; and despite the fourfold higher peak infusion rate in CHF patients, the peak increase in +dP/dt was still higher in normals.

Intracoronary dobutamine was not associated with adverse effects. However, in normal subjects IC dobutamine infusion at rates > 50 μg/min was often associated with palpitations and an increase in heart rate; whereas in CHF patients, infusion rates up to 200 μg/min were well tolerated and not associated with an increased heart rate. Consequently, the routine titration in normals seldom exceeded 50 μg/min. In CHF patients, the 200-μg/min infusion rate was frequently achieved, and was associated with a clear plateau in the +dP/dt response, suggesting that a maximal physiologic effect had been achieved. By contrast, there was little evidence of a plateauing of the +dP/dt response at the peak infusion rates generally achieved in normals.

Dobutamine binding affinity. Dobutamine competition curves for the IPIN binding site were steep and fit best in all cases by a 1-site model. The dissociation constant (K_d) for dobutamine in five patients with severe end-stage CHF (1.9±0.3 μM) was not different from that in five subjects without evidence of cardiac dysfunction (1.6±0.3 μM; P = NS). Likewise, the K_d for IPIN binding was identical in the two groups (normals, 24±4 pM; CHF, 20±2 pM; P = NS).

Hemodynamic effects of IC dobutamine infusion (Fig. 3). In normal subjects, IC dobutamine infusion at 25 μg/min caused no change in heart rate, mean arterial pressure or systemic vascular resistance. In CHF patients, IC dobutamine at 25 μg/min caused no change in heart rate, mean arterial pres-

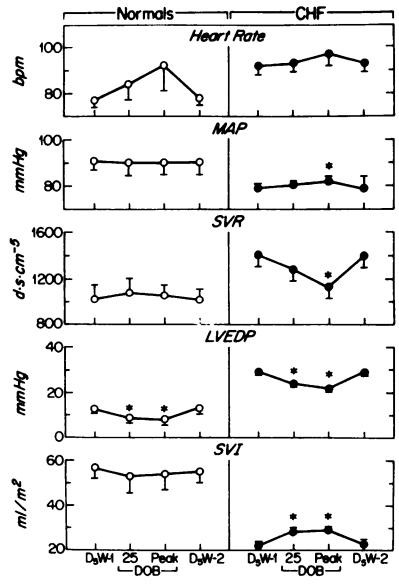

Figure 3. Hemodynamic effects of IC dobutamine infusion to normal subjects (n = 8) and patients with CHF (n = 24). Depicted are the responses to the 25-μg/min infusion, and the peak infusion received by each patient. *$P < 0.01$ vs. D_5W-1 and D_5W-2.

sure, or systemic vascular resistance; and at the peak infusion rate evaluated, there was a small increase in mean arterial pressure (+4±1 mmHg, $P < 0.02$ vs. D_5W) and a small decrease in systemic vascular resistance (−287±76 dyn-s-cm^{-5}; $P < 0.01$).

The 25-μg/min infusion rate caused −4±1 and −5±1 mmHg decreases in left ventricular end-diastolic pressure in normals and CHF patients, respectively ($P < 0.0025$ for both). By contrast, stroke volume index (+6±1 ml/m^2; $P < 0.001$) and stroke work index (+6±1 g.m/m^2; $P < 0.001$) were increased by IC dobutamine (25 μg/min) in CHF patients, but not in normal subjects (P = NS for both) (Figs. 3 and 4). All hemodynamic effects returned to control values during repeat D_5W infusion.

Effect of IC milrinone on the response to IC dobutamine. In nine CHF patients, the initial IC dobutamine infusion was followed by the IC infusion of milrinone, and subsequently, by a second infusion of dobutamine. The initial IC dobutamine infusion (mean infusion rate, 61±16 μg/min) resulted in an increase in +dP/dt in all subjects (mean increase, 52±11%; $P < 0.002$ vs. D_5W) (Fig. 5). There was no significant change in heart rate, mean arterial pressure, or systemic vascular resistance, but left ventricular function was improved as indicated by an increase in stroke work index and a decrease in left ventricular end-diastolic pressure ($P < 0.01$ vs. D_5W for both) (Fig. 6). After discontinuation of dobutamine, +dP/dt, stroke work index and left ventricular end-diastolic pressure all returned to within 5% of the initial D_5W baseline values (Figs. 5 and 6).

Intracoronary milrinone infusion resulted in a 42±6% increase in +dP/dt (Fig. 5) which was associated with significant improvement in left ventricular function as evidenced by an increase in stroke work index and a decrease in left ventricular end-diastolic pressure ($P < 0.01$ for both) (Fig. 6), but no significant change in heart rate, mean arterial pressure or systemic vascular resistance. Immediately following discontinuation of IC milrinone, IC dobutamine was infused again at the same rate as before IC milrinone, and +dP/dt and hemodynamics were measured between 3.5 and 5.0 min later. In every patient, the increase in +dP/dt during the second dobutamine infusion was greater than during the first infusion (mean increase, 74±10%; $P < 0.003$ vs. first dobutamine infusion), and also, was greater than during the immediately preceding IC milrinone infusion ($P < 0.001$) (Fig. 5). Heart rate, mean arterial pressure and systemic vascular resistance were not significantly different than during the first dobutamine or milrinone infusions (P = NS for all). Compared with the hemodynamic effects of the immediately preceding IC milrinone infusion, left ventricular end-diastolic pressure was lower ($P < 0.05$) and stroke work index was higher ($P < 0.05$), indicating a further improvement in left ventricular function (Fig. 6).

In five other CHF patients, the effect of a second IC dobutamine infusion was evaluated without an intervening IC milrinone infusion (Fig. 5). Left ventricular +dP/dt increased from 643±98 to 895±129 mmHg/s ($P < 0.003$) during the first dobutamine infusion (mean infusion rate, 65±15 μg/min),

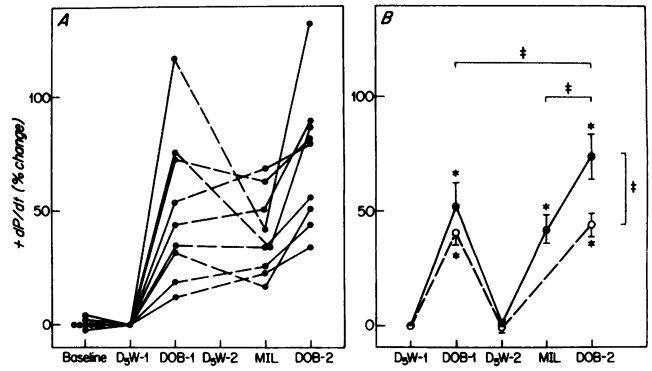

Figure 5. The effect of the phosphodiesterase inhibitor, milrinone, on the +dP/dt response to IC dobutamine infusion in 9 patients with CHF. (*A*) Individual patient responses to IC dobutamine (DOB-1), IC milrinone (MIL), and a second IC dobutamine infusion (DOB-2) immediately after IC milrinone. (*B*) Group mean data for the +dP/dt responses of the nine patients depicted in *A* (●), and five other patients with CHF (○) who received two successive IC infusions of dobutamine (DOB-1 and DOB-2) without an intervening infusion of milrinone. *$P < 0.01$ vs. D_5W-1 and D_5W-2; and ‡$P < 0.01$.

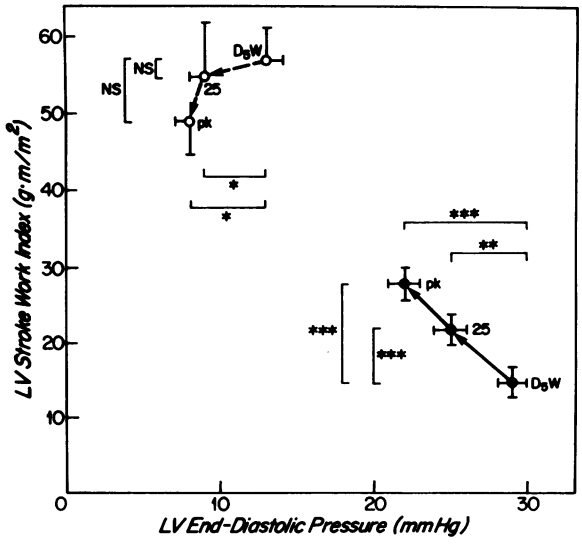

Figure 4. Effects of IC dobutamine infusion on left ventricular pump function in patients with (●) and without (○) CHF. As in Fig. 3, the effects of the 25-μg/min (25) and peak (pk) infusion rates are depicted. *, ** and *** = $P < 0.01$, 0.001, and 0.0001 vs. D_5W, respectively.

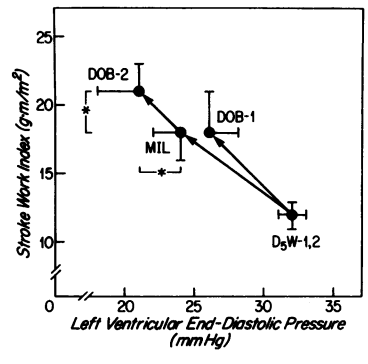

Figure 6. The effect of the phosphodiesterase inhibitor milrinone on the change in left ventricular pump function caused by IC dobutamine infusion in the nine patients with CHF depicted in Fig. 5. After IC milrinone infusion, IC dobutamine (DOB-2) caused a significantly greater decrease in left ventricular end-diastolic pressure and increase in stroke work index ($P < 0.05$ for both) than did milrinone (MIL) or dobutamine alone (DOB-1).

and returned to 641±96 mmHg/s after the infusion was stopped. The +dP/dt increased to 926±148 mmHg/s (P = NS vs. first dobutamine infusion) in response to a second IC dobutamine infusion at the same infusion rate as the first infusion.

Relationship between plasma catecholamines and the response to IC dobutamine. Supine resting plasma norepinephrine and epinephrine concentration sampled during the initial D_5W control period were 142±32 and 54±19 ng/liter in normal subjects. In CHF patients, norepinephrine (837±208 ng/liter; range 136–3972 ng/liter) and epinephrine (139±29 ng/liter; range, 31–309 ng/liter) were markedly elevated at rest and significantly higher than in normals ($P < 0.001$ for norepinephrine; $P < 0.03$ for epinephrine). In CHF patients, neither norepinephrine nor epinephrine correlated significantly with heart rate, mean arterial pressure, left ventricular end-diastolic pressure, stroke volume index, cardiac index, or stroke work index ($P > 0.10$ for all).

The increase in +dP/dt in response to IC dobutamine (25 μg/min) ranged from 49 to 148% in normals, and from 0 to 73% in CHF patients; and was inversely related to resting plasma norepinephrine ($r = -0.653$, $P < 0.001$ for all patients; $r = 0.615$, $P < 0.001$ for CHF patients only) (Fig. 7). There was no significant relationship between resting epinephrine and the +dP/dt response to IC dobutamine ($r = -0.300$; $P = 0.260$ for all patients; $r = -0.378$; $P = 0.230$ for CHF patients only).

In 11 of the patients with CHF, plasma norepinephrine was determined at each IC dobutamine infusion rate. Baseline plasma norepinephrine (518±97 ng/liter) was stable over the 30-min control period (c-1, c-2, and D_5W), but decreased progressively during IC dobutamine infusion, with a maximum decrease of 160±31 ng/liter ($P < 0.001$ vs. D_5W) at the peak dobutamine infusion rate (Fig. 8). Norepinephrine returned to within 5% of the initial baseline value 10 min after discontinuation of IC dobutamine infusion ($P < 0.001$ vs. peak dobutamine) (Fig. 8).

Discussion

The density of beta adrenergic receptors is decreased in the myocardium of patients with severe CHF (2–4). However, the physiologic consequence of this decrease in total beta adrenergic receptor number is not evident *a priori* since regulation of the beta adrenergic pathway can also involve alterations in the affinity of beta adrenergic receptors for agonist and the degree of coupling to adenylate cyclase (19, 20), and may be influenced importantly by such factors as the presence of spare beta adrenergic receptors in excess of the number required for a maximal response and by the efficiency of the coupling of beta adrenergic receptors to contraction (21). The use of the intracoronary infusion method allowed an examination of the positive inotropic effect of dobutamine over an almost 100-fold concentration range, and achievement of a steady state myocardial drug concentration at least 10-fold greater than that achievable during intravenous drug infusion. In CHF patients, a clear plateau in the dose-response curve was achieved over a fourfold dose range, and therefore, we were able to estimate the EC_{50} for dobutamine's positive inotropic effect. Whereas maximal concentrations generally can be achieved during in vitro studies, we are unaware of previous studies that have been able to assess maximal or near-maximal responses to positive inotropic agents in vivo in man. In normal subjects, we were not able routinely to achieve maximal infusion rates of dobutamine due to the development of marked palpitations and tachycardia at infusion rates higher than 50 μg/min. Since tachycardia, per se, can influence +dP/dt, we restricted our infusion rates to doses that did not significantly increase heart rate. From inspection of Fig. 2 *A*, it can be seen that the EC_{50}'s for dobutamine's effect on +dP/dt are very similar in CHF patients and normal subjects. This apparent lack of a rightshift in CHF patients is strengthened by the fact that a maximal effect was not achieved in normal individuals, and therefore the true EC_{50} in normal subjects is probably further to the right that that estimated from Fig. 2 *A*. Only one prior in vivo study has compared the positive inotropic responses to dobutamine in patients with and without CHF (3). Our data confirm the finding in that study of a substantial decrease in the inotropic response to dobutamine in CHF patients, and in addition, provide new information regarding the relative importance of a decrease in the maximal amplitude of response versus a shift in EC_{50}. The radioligand binding data further suggest that the decreased efficacy of dobutamine is not due to a decrease in beta adrenergic receptor affinity for dobutamine in CHF patients. Our findings are also consistent with in vitro observations by Bristow et al. in myocardium from CHF patients showing that the dose-response curves for isoproterenol-stimulated adenylate cyclase activation and contraction were shifted downward, without a rightward shift in EC_{50} (2).

A decrease in the maximal physiologic response to dobutamine (i.e., decreased efficacy) without a change in EC_{50} (i.e., potency) may be due to a decrease in beta adrenergic receptor number, other abnormalities of the beta adrenergic receptor pathway, such as a decreased quantity and/or function of the

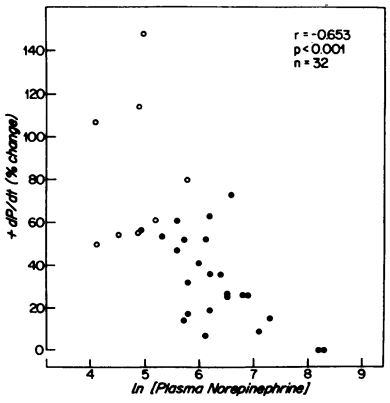

Figure 7. Relationship between resting plasma norepinephrine concentration and the increase in +dP/dt caused by a 25-μg/min IC infusion of dobutamine in patients with (n = 24, ●) and without (n = 8, ○) CHF.

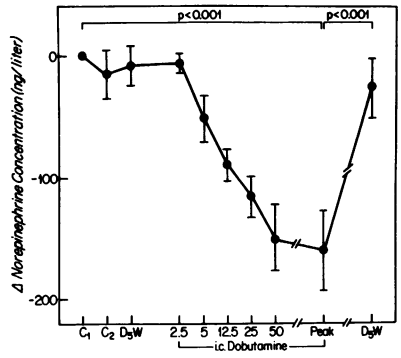

Figure 8. Effect of IC dobutamine infusion on plasma norepinephrine concentration in 11 patients with CHF. Control (C_1) plasma norepinephrine was 518±97 ng/liter. The data are depicted as absolute change in norepinephrine concentration compared to C_1.

guanine nucleotide regulatory protein (N_s) (22–24), or an absolute reduction in the ability of contractile elements to respond to cyclic AMP or calcium. The latter possibility is unlikely to explain our data, since (*a*) pretreatment with the phosphodiestase inhibitor milirinone substantially increased the positive inotropic response to dobutamine in CHF patients, thus indicating that a further positive inotropic response is possible; and (*b*) in vitro and in vivo studies have shown a selective loss of the biochemical and contractile responses to beta adrenergic receptor stimulation without a reduction in the responses to digitalis, calcium or cyclic AMP (3, 5–7). Although it is not necessary to postulate a defect other than a decrease in beta adrenergic receptor density, we can not exclude the role of an abnormality in the quantity and/or function of N_s or the catalytic unit of adenylate cyclase. However, in vitro data indicate that the contractile responses to histamine and forskolin are normal in myocardium from CHF patients (2, 6, 7).

The observation that a decrease in maximal response to dobutamine is not associated with a rightshift in the dose-response relationship suggests that operationally there is little or no receptor reserve in the beta adrenergic response to dobutamine. As stressed by Kenakin (21), this observation does not necessarily indicate the absence of "spare" beta adrenergic receptors, and may not be generalized for other agonists. However, this conclusion is consistent with that of Bristow et al., which was based on the agreement between the magnitudes of decrease in beta adrenergic receptor density and maximum isoproterenol-stimulated contraction and adenylate cyclase activation in vitro in CHF myocardium (2). Taken together, these observations indicate that the reduced inotropic response to beta adrenergic stimulation can not be overcome fully even by high concentrations of dobutamine.

Radioligand binding and physiologic data suggest that dobutamine may interact not only with beta-1 adrenergic receptors, but also with alpha-1 and beta-2 adrenergic receptors (25–27). However, in rat myocardium the interaction with beta-2 adrenergic receptors is of low affinity and not influenced by guanine nucleotides (25). Thus, although physiologically coupled beta-2 adrenergic receptors are present in human myocardium (4), it is uncertain whether dobutamine acts as an agonist at these receptors. The role of alpha-1 adrenergic receptors in mediating a positive inotropic response in human myocardium is also unresolved (28, 29). However, preliminary data from our laboratory fail to show a significant effect of the alpha adrenergic antagonist phentolamine on the +d*P*/dt response to IC dobutamine infusion in CHF patients (30). Thus, we feel that the action of dobutamine in this study is primarily due to stimulation of myocardial beta adrenergic receptors. In view of the recent demonstration of a significant beta-2 adrenergic receptor-mediated contractile response in human myocardium, particularly in the presence of beta-1 adrenergic receptor desensitization due to CHF (4), it is possible that the effect of dobutamine does not reflect the full beta adrenergic potential (i.e., beta-1 plus beta-2) of the myocardium in such patients.

These data provide evidence that the positive inotropic action of the phosphodiesterase inhibitor milrinone in patients with severe CHF is complementary to that of the beta adrenergic agonist, dobutamine, and that the combined effects of milrinone and dobutamine result in a significantly greater improvement in hemodynamic performance than either alone. Thus, in every subject the increase in +d*P*/dt with IC dobutamine immediately following IC milrinone was greater than that caused by either dobutamine or milrinone alone. Milrinone acts primarily by inhibition by phosphodiesterase (31, 32), thereby causing an increase in the accumulation of myocardial cyclic AMP. It is probable that milrinone potentiated the positive inotropic response to dobutamine by causing a greater accumulation of cyclic AMP in response to any given degree of beta adrenergic receptor stimulation by dobutamine. Our findings are consistent with in vitro data. For instance, the positive inotropic effect of norepinephrine on rabbit papillary muscle is potentiated by the phosphodiesterase inhibitor theophylline (33). Likewise, the positive inotropic response of guinea pig papillary muscle to isoproterenol is potentiated when isoproterenol is given at the peak of a minimally effective concentration of milrinone or after prolonged incubation with milrinone (34). Only one other study has addressed this important issue in vivo in patients with CHF (35). Our data and conclusion are consistent with those of Gage et al. (35) who showed that the +d*P*/dt response to intravenous dobutamine was greater in the presence of intravenous amrinone. In addition, the IC infusion method allows two further conclusions. First, the effect of milrinone is due to an increase the maximal amplitude of response to dobutamine, since the 74% increase in +d*P*/dt to dobutamine after milrinone was substantially and consistently greater than the 52% maximal response to dobutamine alone. Second, due to the design of our study, the further improvement in left ventricular pump function caused by IC dobutamine following IC milrinone, as reflected by the relationship between stroke work index and left ventricular end-diastolic pressure, can be attributed entirely to the direct actions of the two agents on myocardial systolic and/or diastolic (36) function. Although Gage et al. (35) demonstrated that the +d*P*/dt response to dobutamine was greater in the presence of intravenously administered amrinone, the actual contribution of this positive inotropic action to the overall hemodynamic effect of the combination of systemic dobutamine and amrinone could not be determined, since systemic amrinone also exerts a substantial vasodilator action which, in and of itself, could cause a significant hemodynamic improvement (37).

We used +d*P*/dt as a measure of the positive inotropic effect of dobutamine. This measurement can also be influenced by changes in preload, afterload, and heart rate. However, by using the direct intracoronary infusion method, we were able to avoid significant changes in heart rate or mean arterial pressure. Interestingly, left ventricular end-diastolic pressure decreased during intracoronary infusion of both dobutamine and milrinone. Since, as shown in Fig. 1 *A*, the intracoronary infusion method avoids significant peripheral levels of the infused drug, this effect may be due to a direct drug-induced improvement in myocardial relaxation (36), possibly due to increased cAMP levels, and/or to improved forward pump function. A drug-induced decrease in left ventricular end-diastolic pressure theoretically could have attenuated the apparent magnitude of the positive inotropic response. However, it is unlikely that this change in left ventricular end-diastolic pressure significantly affected the measurement of +d*P*/dt in this study, since in a prior study of CHF patients with a comparable degree of hemodynamic compromise, we found that lowering left ventricular end-diastolic pressure to a similar degree with nitroprusside had no

significant effect on +dP/dt (11). Alternatively, it has been shown that at least one class of positive inotropic agents, the phosphodiesterase inhibitors, can increase left ventricular volume despite lowering end-diastolic pressure (38). Since we did not assess the effect of dobutamine on left ventricular volume, we cannot exclude the possibility that drug-induced changes in preload contributed to the observed changes in +dP/dt.

The mechanism responsible for a decrease in myocardial beta adrenergic receptors and responsiveness in CHF is not known. By analogy to a large number of in vitro studies, it has been suggested that beta adrenergic desensitization in heart failure is a result of increased sympathetic nervous system activity (39). This hypothesis, however, has not been tested directly in patients. Fowler et al. noted an inverse correlation between beta adrenergic receptor density and the concentration of catecholamines in the coronary sinus of CHF patients (3). We previously observed that the positive inotropic response to intravenous dobutamine was attenuated relative to the effect of milrinone, and that the attenuation was most marked in the CHF patients in whom hemodynamic dysfunction and plasma norepinephrine elevation were more marked (14). In a small group of CHF patients we previously observed a weak inverse relationship between circulating norepinephrine and the +dP/dt response to IC dobutamine in CHF patients (9). In the present, larger population this correlation continues to be significant, and therefore further supports a role of sympathetic nervous system activation in determining the physiologic beta adrenergic responsiveness of CHF patients. The relatively weak correlation may reflect the indirect relationship between circulating catecholamines and sympathetic nervous system activity, or may be due to the participation of other factors in the regulation of beta adrenergic receptor responsiveness.

In contrast to some prior studies, we did not find a significant correlation between circulating catecholamines and several measures of hemodynamic function. This is most likely a result of the relatively homogeneous hemodynamic status of our CHF patients. However, a novel observation in this study is that IC dobutamine caused a significant and rapid decrease in circulating plasma norepinephrine. Since the hemodynamic effects of IC dobutamine can only be attributed to improved myocardial function, this finding strongly suggests that despite the poor correlation between catecholamines and hemodynamic measures for the whole group, there is an important relationship between acute changes in hemodynamic function and sympathetic nervous system tone in individual patients.

The findings of this study have potential clinical importance. First, they indicate that in patients with severe CHF, the hemodynamic consequences of myocardial beta adrenergic receptor desensitization can not be overcome fully by high doses of dobutamine. Second, the ability of milrinone to increase the maximal amplitude of response to dobutamine suggests that an important action of phosphodiesterase inhibitors such as milrinone may be to ameliorate, partially, the consequences of beta adrenergic receptor desensitization; and provides a rationale for the combined administration of a beta adrenergic agonist and a phosphodiesterase inhibitor to patients with severe CHF. Finally, since there is an inverse relationship between circulating catecholamines and the severity of the physiologic desensitization to dobutamine's positive inotropic effect, the data support the notion that therapies that reduce sympathetic nervous system activity and/or reduce beta adrenergic receptor stimulation could lead to a decrease in beta adrenergic desensitization (40).

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