Assessment of the β-adrenergic receptor pathway in the intact failing human heart: progressive receptor down-regulation and subsensitivity to agonist response

MICHAEL B. FOWLER, M.D., JEFFREY A. LASER, M.D., GREGG L. HOPKINS, M.D., WAYNE MINOBE, B.S., AND MICHAEL R. BRISTOW, M.D. PH.D.

ABSTRACT We developed methods for identifying β-adrenergic receptors in human right ventricular endomyocardial biopsy tissue with the radioligand (−) [125I]iodocyanopindolol (ICYP). Specific ICYP binding in a crude, high-yield membrane preparation derived from endomyocardial biopsy tissue was high (specificity > 90%), of high affinity (Kᵦ around 20 pM), saturable and stereospecific for the (−) vs the (+) isomer of isoproterenol. Subjects with mild-moderate and severe biventricular dysfunction had respective decreases in β-adrenergic receptor density of 38.2% and 57.7% when normalization methods were averaged, with no significant differences in ICYP dissociation constant. A subgroup of subjects was subdivided by left ventricular ejection fraction (LVEF) into those with mild cardiac dysfunction (LVEF < 0.50 > 0.40) and severe heart failure (LVEF < 0.20) and given graded sequential infusions of dobutamine and calcium gluconate. Those with severe cardiac dysfunction had marked impairment of the dobutamine dP/dt and stroke work index response, whereas these responses to calcium did not differ in the two groups. These data indicate that in the intact human heart (1) endomyocardial biopsy may be used for direct analysis of β-adrenergic receptors, (2) heart failure-associated myocardial β-adrenergic down-regulation begins with mild-moderate ventricular dysfunction, (3) reduction in myocardial β-receptor density is related to degree of heart failure, and (4) β-receptor down-regulation is associated with pharmacologically specific impairment of the β-agonist-mediated contractile response.

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β-ADRENERGIC RECEPTORS represent an important inotropic regulatory mechanism available to myocardial cells. Previous studies in tissue removed from human hearts indicate that β-receptor regulatory changes may lead to β-adrenergic subsensitivity of the severely failing human heart.1-5 These receptor regulatory changes may explain decreased responsiveness to both endogenous and exogenous β-agonist stimulation.1-7

Previous measurements of β-adrenergic receptor density in human ventricular myocardium have required gram quantities of tissue to identify receptors.1,5-6 Ventricular myocardium is generally available in these quantities only in explanted hearts removed at the time of transplantation1-4 or in papillary muscles amputated at the time of mitral valve replacement.5 This has limited the investigation of ventricular myocardial β-adrenergic receptors to surgically removed tissue taken from nonfailing transplant donor hearts, severely failing transplant recipient hearts, or subjects with valvular heart disease.

Milligram quantities of human endomyocardial tissue can be obtained from the intact heart with relative ease and safety in the cardiac catheterization laboratory,8 and transvenous right ventricular endomyocardial biopsies are routinely performed on an outpatient basis at many institutions.9 The technique of endomyocardial biopsy has already proved useful in the evaluation

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of myocarditis, transplant rejection, and anthracycline cardiotoxicity, as well as in some metabolic studies. The recent introduction of iodinated radioligands with high specific activity and high affinity for β-adrenergic receptors, such as (−)[125I]iodocyanopindolol (ICYP), has made possible the measurement of β-adrenergic receptor density in milligram quantities of tissue. Such measurements are important because they would make available a spectrum of patients with varying degrees of myocardial dysfunction for receptor and other biochemical analyses. More important, such measurements would allow for direct assessment of the β-adrenergic pathway in the intact human heart, as compared with examination of hearts that have been subjected to anesthesia, surgical procedures, and a variety of preoperative systemic influences.

We report our initial results of the assessment of the β-adrenergic receptor pathway in the intact human heart with tissue removed by endomyocardial biopsy and measurement of the functional response to the β-agonist dobutamine. The data indicate that β-receptors may be directly identified in endomyocardial biopsy tissue, that β-adrenergic receptor down-regulation occurs in hearts with mild to moderate dysfunction as well as in severely failing hearts, that in heart failure the degree of receptor down-regulation is related to the degree of cardiac dysfunction, and that β-receptor down-regulation is associated with a specific blunting of the contractile response to β-agonists.

Materials and methods

Patients. The study population consisted of 38 consecutive subjects who had undergone right ventricular biopsy (n = 38), cardiac catheterization (n = 38), and measurement of ejection fraction (n = 37) by either contrast (n = 18) or radionuclide (n = 19) techniques between March 1, 1983, and March 1, 1985, at either Stanford University Hospital or the University of Utah Medical Center. Five patients with normal ventricular function were evaluated in an investigation of the cardiotoxicity of an anthracycline antibiotic analog, 4′-epidoxorubicin. These subjects all had low doses of 4′-epidoxorubicin (<400 mg/m²) and none had a biopsy score of greater than 2.0 on the Billingham scale of anthracycline damage. Another subject was being treated with a cardiotoxicity-sparing dose schedule of doxorubicin (adriamycin) and had a grade 2.0 biopsy score with normal hemodynamics at rest and exercise at a total cumulative doxorubicin dose of 1050 mg/m². An additional subject with normal ventricular function was evaluated because of apparent familial cardiomyopathy in his offspring and was found to have normal hemodynamics at rest and exercise. Patients with biventricular dysfunction by hemodynamic measurements or by disease etiology included subjects with idiopathic (n = 26), anthracycline (n = 1), anthracycline plus other causes (n = 2), and diabetic cardiomyopathy (n = 1). Twenty-three subjects with ventricular dysfunction underwent left heart catheterization and coronary angiography, and with the exception of the one subject with diabetic cardiomyopathy, none had coronary artery narrowing of greater than 50%. This subject had three-vessel coronary disease, no history of myocardial infarction, and diffuse hypokinesis on ventriculography.

Pathophysiology and natural history—ventricular performance

Patients were excluded from this study if they were older than 60 years, had heart failure secondary to ischemic heart disease by history, electrocardiography, or coronary arteriography, had significant lung disease, or had valvular heart disease exclusive of mild-to-moderate mitral or tricuspid regurgitation thought to be secondary to dilated cardiomyopathy.

Radionuclide ventriculography was performed as previously described. When subjects underwent both contrast and radionuclide ventriculography, the contrast value was used. All subjects had radionuclide ventriculography within 1 week of catheterization. For analysis of data within the three treatment groups, contrast and radionuclide values were considered equal.

Subjects were divided into three groups based on left ventricular ejection fraction (LVEF). Group I consisted of subjects without ventricular dysfunction with an LVEF of 0.50 or greater. Group II included subjects with an LVEF of 0.30 to 0.50 but greater than 0.25. Group III comprised subjects with severe ventricular dysfunction and an LVEF under 0.25. Informed written consent was obtained from all patients, and this research was approved by the institutional review boards at both institutions.

Endomyocardial biopsy. Right ventricular endomyocardial biopsy specimens were obtained from all subjects. In six subjects in group I and two subjects in group II, studies were performed on an outpatient basis. The procedure of right ventricular endomyocardial biopsy has been described in detail elsewhere. Briefly, a No. 9F sheath is inserted percutaneously into the right internal jugular vein under local anesthesia. A 50 cm right ventricular bioptome is then inserted through the sheath into the right ventricle and positioned against the intraventricular septum. In the current study, multiple specimens (two or three) were obtained for morphologic examination by both light and electron microscopy, and three to six specimens were obtained for β-adrenergic receptor analysis.

After biopsy, right heart catheterization was performed through the same sheath with either a No. 7F Courand catheter or a balloon-tipped catheter (Critikon Corp.), with a reduction sleeve on the sheath. Cardiac output was measured by the Fick method. Left heart catheterization, coronary angiography, and left ventricular ventriculography were performed by standard techniques.

Membrane preparation. The biopsy specimen was immediately placed in 10 ml of ice-cold 10 mM Tris, 1 mM EGTA buffer, pH 8.0, and grossly visible fibrous tissue was dissected free. The remaining sample was compiled as an aggregate, blotted dry, and weighed. Tissue weight ranged from 10.6 to 57.7 mg, with a mean ± SD of 25.2 ± 10.2 mg. The samples were placed in 40 ml of ice-cold Tris-EGTA buffer and homogenized in a Polytron (Brinkmann Instruments, Westbury, NY) by three consecutive 3 sec bursts at the maximum setting of 11. One milliliter of ice-cold 2.5M KCl was then added to extract contractile proteins, followed by stirring at 4°C for 15 min. The suspension was then centrifuged at 50,000 g for 15 min and resuspended with a quick burst in the Polytron and recentrifuged and resuspended in the same manner. After a third centrifugation the preparation was suspended in the final assay buffer at a final concentration of 1 to 3 mg of original tissue to 1 ml of buffer and used for receptor binding assays.

For certain characterization procedures requiring larger quantities of tissue (linearity with protein, kinetics, within-sample variance, membrane marker measurements), 80 to 270 mg of right ventricular endomyocardium was removed from the distal
septum of five hearts removed at transplantation. Four of these hearts were from recipients with idiopathic dilated cardiomyopathy and severe end-stage biventricular failure, and one was from a prospective donor. The preparation of this tissue was identical to that of biopsy specimens, after which the crude membranes were stored at $-70^\circ$C in 50 mM Tris, 250 mM sucrose, 1 mM EGTA buffer, pH 7.5, at a protein concentration of 5 mg/ml. The material was then thawed and used for receptor assays as described for biopsy material. In these same hearts, a crude membrane fraction for receptor and membrane marker assays was also prepared from 5 g aliquots of left and right ventricular free walls.

In seven additional explanted hearts (three prospective non-failing donor hearts and four from transplant recipients with idiopathic dilated cardiomyopathy and severe biventricular failure), 5 g aliquots of left and right ventricular free wall were removed and membranes were prepared and stored as described above. These and the five other explanted heart preparations were used to assess receptor functionality and membrane yield in nonfailing compared with failing ventricular chambers.

**Receptor assay.** The radioligand ICYP was used for identifying $\beta$-adrenergic receptors. ICYP was obtained from Amer sham (Arlington Heights, IL) and had a specific activity of 1925 to 2050 Ci/mmol at the time of iodination. The assay buffer was 20 mM Tris, 150 mM NaCl, and 1 mM ascorbate, pH 7.5 (at room temperature). For measurement of receptor density, duplicate tubes containing seven increasing concentrations of ICYP from 3 to 150 pM with and without 1 $\mu$M (-) propranolol in a total volume of 150 $\mu$l were prepared. The assay was begun with the addition of 300 $\mu$l of membrane preparation. For steady-state measurements the assay was incubated for 120 min at 30°C, followed by dilution with 5 ml of room-temperature buffer and vacuum filtration through 1 $\mu$m fiberglass filters (Gelman Science, Ann Arbor, MI). Each filter was then washed with 20 ml of Tris/NaCl buffer at a flow rate of 2 ml/sec. The filters were then dried and counted on a Micromedic Systems, Inc. (San Clemente, CA) gamma counter at an efficiency of 69%.

**Receptor binding.** Maximum bound ICYP ($B_{\text{max}}$) and the radioligand dissociation constant ($K_D$) were determined by analysis of saturation isotherm data. Nonlinear least-squares regression analysis of one form of Michaelis-Menten equation:

$$\text{Bound radioligand} = \frac{B_{\text{max}} X}{(K_D + X)}$$

where $X$ = radioligand concentration, was performed as previously described. Specifically bound ICYP was derived by subtraction of the linear fit of the nonspecific binding curve from the total binding curve, as previously described. Linear plots were also performed but were used only for data inspection. In calculating the specific activity of ICYP, ICYP was assumed to undergo transformation into non-$\beta$-receptor active breakdown products in direct relation to radioisotope decay.

In kinetic experiments, the dissociation rate constant ($k_2$) was determined by a nonlinear least-squares fit of the equation $B_p = B_0 e^{-k_2 t}$, where $B_0 = $ specifically bound ICYP remaining from an initial amount $B_0$. The association rate constant ($k_1$) was determined from the formula $k_1 = (k_{\text{obs}} - k_2)/[\text{ICYP}]$, with $k_{\text{obs}}$ determined from a nonlinear least-squares fit of the equation $B_p = B_{eq} (1 - e^{-k_{\text{obs}} t})$, where $B_{eq} = B_0$ at equilibrium.

**Protein, adenylate cyclase, and 5’ nucleotidase measurements.** Protein measurements were made by the Peterson modification of Lowry’s method and by the method described by Bradford. In the Peterson modification, precipitation of protein by trichloroacetic acid was omitted. Standard curves were run with bovine serum albumin for the Lowry method and gamma globulin for the Bradford method. Adenylate cyclase was measured in myocardial membrane preparations as previously described, and 5’ nucleotidase was measured by the method of Ipata.

**Contractile response to dobutamine and calcium.** Eighteen male patients who were being evaluated for suspected idiopathic dilated cardiomyopathy consented to a substudy consisting of evaluation of the hemodynamic response to dobutamine or calcium. These subjects were examined in the postabsorptive state at the time of routine cardiac catheterization. The median age of these subjects was 41 years (range 25 to 60). All had been scheduled for routine diagnostic right ventricular endomyocardial biopsy, left ventricular angiography, and right and left heart hemodynamic measurements. Sixteen patients were shown to have minimal or no coronary artery disease by coronary arteriography performed at the time of the study or at coronary arteriography performed at another institution within the previous 12 months. In two patients, both under 28 years old with global left ventricular hypokinesis, coronary arteriography was not performed. The patients were divided into two groups according to their resting LVEF. Group A consisted of seven patients with no or mild heart failure and an LVEF greater than 0.40; subgroup B consisted of 11 patients with moderate-to-severe heart failure and an LVEF under 0.30 (range 0.10 to 0.26). After right ventricular biopsy and procurement of tissue for $\beta$-receptor binding studies as described above, a left ventricular angiogram was obtained in the 30 degree right anterior oblique projection in all the patients. LVEF was calculated by digitizing the end-systolic and end-diastolic images with a dedicated computer system. A Gooldale-Lubin catheter was then placed in the coronary sinus or in a stable position in the right atrium to achieve a controlled paced heart rate during the study. A Swan-Ganz catheter was used to measure right atrial, pulmonary arterial, and pulmonary arterial wedge pressure, as well as cardiac output by the thermodilution method using triplicate 10 ml injections of 5% dextrose at room temperature. A No. 5 catheter-tip micromanometer (Millar Instruments) inserted via a long sheath (No. 8F Cook catheter) was used to measure left ventricular pressure, left ventricular end-diastolic pressure, and peak negative and peak positive dP/dt. All pressures were recorded on paper with a Honeywell-Meddars chart recorder.

Baseline and subsequent hemodynamic data were obtained during atrial pacing between 110 and 115 beats/min. In two patients with atrial fibrillation, heart rate was controlled in the same range with right ventricular pacing. Hemodynamic measurements were repeated (at the same paced heart rate) 5 min after starting dobutamine infusion of 2.1, 4.2, and 8.2 $\mu$g/kg/min. After hemodynamic measurements had been obtained, the pacemaker was turned off to record spontaneous heart rate during each infusion dose. Twenty to 30 min after the final dobutamine infusion, a second set of “baseline” hemodynamic measurements was recorded before and after three infusions of 10% calcium gluconate infused for 2 min at 2.5, 5, and 10 mg/kg/min. Whole blood “on-line” ionized calcium measurements were made in the first six patients studied before and at the end of each 2 min infusion by an ion-specific calcium electrode (Applied Medical Electronics, Palo Alto, CA). In all the patients arterial blood was drawn immediately before and after each set of hemodynamic measurements, and the serum was stored for subsequent analysis of ionized calcium values on the same instrument; the ionized calcium level was calculated from the mean value of these two samples. After the 2 min 5 and 10 mg/kg/min infusions of calcium gluconate, hemodynamic measurements were made at the previous infusion rate to prevent an excessive rise in ionized calcium levels.

**Statistical methods.** Significant differences between group means were assessed by one-way analysis of variance (ANOVA), followed by the Newman-Keuls multiple range test.
when more than two groups were compared. Results were considered statistically significant at p(F) < .05 by ANOVA combined with p < .05 for the Newman-Keuls test. All values are expressed as mean ± SD or mean ± SEM.

Dose-response curves to calcium and dobutamine were constructed by subtracting baseline values and analyzing the data points by a multivariate analysis of variance analog of a repeated-measures analysis of variance, as previously described.21

Results

Assay characterization
Membrane preparation. The crude membrane preparation used for endomyocardial biopsy tissue has been extensively characterized in gram quantities of starting material in canine22 and human ventricular myocardium.1,21 Values for the sarcosomal membrane markers basal adenylate cyclase activity, fluoride- and forskolin-stimulated adenylate cyclase activities, 5' nucleotidase activity, and β-adrenergic receptor density are given in table 1 for both left and right ventricular free wall and for right ventricular endomyocardium. “Homogenate” in table 1 refers to right ventricular endomyocardial tissue homogenized and pelleted at 50,000 g and resuspended in assay buffer. In right ventricular endomyocardium there is a 1.7- to 2.0-fold enrichment of the membrane markers β-adrenergic receptor density, basal adenylate cyclase, fluoride-stimulated adenylate cyclase, and 5' nucleotidase into the final preparation. The recovery of β-receptors in right ventricular endomyocardial crude membranes from the homogenate was 91.5%.

The distribution of β-adrenergic receptors, isoproterenol-stimulated adenylate cyclase activity, and various membrane markers in right ventricular endomyocardium, right ventricular free wall, and left ventricular free wall are also given in table 1. Although β-receptor density and 5' nucleotidase activity are comparable in right ventricular endomyocardium and free wall, adenylate cyclase activities appear to be lower in endomyocardium compared with right ventricular free wall. Left ventricular free wall had a slightly greater β-receptor density than did right ventricular free wall or endomyocardium, but the small difference was not statistically significant. Relative to right ventricular tissue, left ventricular myocardium had statistically greater forskolin- and isoproterenol-stimulated cyclase activities but similar 5' nucleotidase activity.

Radioligand binding. Figure 1 demonstrates that specifically bound ICYP is linear with respect to added protein between concentrations of 3 and 443 μg/ml. In figure 2, specifically bound ICYP demonstrates stereospecificity for competition by the (-) isomer vs the (+) isomer of isoproterenol. The rank order of potency for various agonists given in figure 2 is: (-) isoproterenol > epinephrine = norepinephrine, which is the order of potency for a predominantly β2-receptor population. Results similar to those shown in figures 1 and 2 were obtained in two other preparations.

Kinetic data from a representative experiment are given in figure 3 and reveal rather slow onset kinetics, with steady state achieved by 105 min. The offset kinetics are extremely slow, as shown in the left-hand panel of figure 3. The Kp, determined from the kinetic

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>ICVP</th>
<th>Adenylate cyclase (pmol/min/mg)</th>
<th>5' Nucleotidase (nmol/min/mg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Bmax (fmol/mg)</td>
<td>Basal activity</td>
<td>Net stimulation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Isoproterenol</td>
<td>Fluoride</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVE</td>
<td>H</td>
<td>±4.6</td>
<td>±2.1</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>±9.2</td>
<td>±4.2</td>
</tr>
<tr>
<td>RV</td>
<td>M</td>
<td>±11.6</td>
<td>±5.7</td>
</tr>
<tr>
<td>LV</td>
<td>M</td>
<td>±6.2</td>
<td>±8.5</td>
</tr>
</tbody>
</table>

H = homogenate; M = crude membranes; RVE = right ventricular endomyocardium; RV = right ventricular; LV = left ventricular; ISO/F = isoproterenol/fluoride ratio.

*p < .05 vs RVE M; *p < .05 vs RV M.
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FIGURE 1. Relation of specifically bound ICYP to protein concentration in right ventricular (RV) endomyocardium. Specifically bound ICYP in counts per minute (CPM), defined as binding under steady-state conditions (see Methods) of 200 pM ICYP that is inhibited by 1 µM (-) propranolol, is plotted on the ordinate; protein concentration (µg/ml), determined by the Bradford method (see Methods), is plotted on the abscissa.

Data shown in figure 3 ($k_2/k_1$) was 2.7 pM, which was similar to the $K_D$ determined by steady-state methods in this membrane preparation (3.9 pM). A linear plot of these data is shown in the right-hand panel of figure 3. For three experiments in three different preparations the mean $k_2/k_1$ value was 3.7 ± 1.3 (SD) pM compared to a $K_D$ of 7.6 ± 4.1 pM determined by steady-state methods.

Representative saturation binding curves for preparations from groups I, II, and III are shown in figure 4. Specific binding (circles) is saturable and of a high percentage (>75% of total binding) at low concentrations of ICYP. Scatchard plots are given in the insets of figure 4 and reveal a single class of binding sites within the range of the ICYP concentrations examined, with linear plots of bound/free vs bound. For all assays protein concentrations ranged from 7 to 81 g/ml as measured by the Lowry method and 5 to 40 g/ml by the Bradford method (respective means ± SD, 38.2 ± 22.0 and 20.2 ± 11.9).

To assess within-individual variance, in one heart six pieces of right ventricular endomyocardium weighing 96.5 to 132 mg were removed and assayed by ICYP binding. The six pieces yielded coefficients of variation (SD/mean × 100) of 11.9% and 14.4% for ICYP $B_{max}$ normalized, respectively, to gram wet weight and milligrams of protein by the Lowry method.

Experimental groups and descriptive clinical data. Of the 38 subjects examined, 36 had receptor binding assays that could be evaluated. In one group III subject, inadequate tissue (<10 mg) was obtained despite repeated attempts at biopsy, and in another group III subject, specific binding was too low (<200 cpm at 12.5 pM) for analysis despite obtaining 37 mg of tissue. The ages, genders, and NYHA functional classes of these 36 subjects are given in table 2. There were no differences in age, which ranged from 38 to 59 in group I, 28 to 60 in group II, and 20 to 60 in group III. Group III had a significantly greater number of men than group I, and NYHA functional class increased progressively in the three groups.

Hemodynamics. As shown in table 3, hemodynamic data divide the groups into three distinct subsets. Mean right atrial pressure, mean pulmonary wedge pressure, cardiac index, and LVEF all separate group III from

![Graph](image-url)

FIGURE 2. Competition of the (-) and (+) isomers of isoproterenol (respectively labeled L and D), norepinephrine, and epinephrine with ICYP in membranes derived from endomyocardial biopsy tissue. ICYP concentration was 50 pM, assay was performed under steady-state conditions as outlined in Methods except that the buffer was 20 mM Tris, 135 mM NaCl, 10 mM MgCl₂, and 1 mM ascorbate, pH 7.5.
group I; mean right atrial pressure, mean pulmonary wedge pressure, and LVEF separate group II from group III; and cardiac index and LVEF separate group I from group II.

β-adrenergic receptor binding. β-receptor binding data are given in table 4. There is a decrease in β-receptor density (B_{max}) as hemodynamic impairment increases from group I to group III. By normalization to protein measured by the Lowry method, receptor density in group II is 44.7% of that in group I, and in group III receptor density is 33.2% of that in group I. The average decrease in β-receptor density by all three normalization procedures (Lowry and Bradford protein measurements and wet weights) is 38.2% in group II and 57.7% in group III. Statistically, group III can be separated from group I by all three normalization procedures, whereas group II is different from group I in the Lowry normalization and group III is different from group II by normalization to gram wet weight.

ICYP dissociation constants did not differ in the three groups (table 4), and in group I B_{max} values in the four women (104.6 ± 22.3 fmol/mg, SEM) did not differ from those in the three men (109.6 ± 14.3 fmol/mg; p = NS).

Data normalization procedures and within-group variability. Of the three normalization procedures given in table 4, respective coefficients of variation (SD/mean) for Lowry protein, Bradford protein, and gram wet weight were .50, .52, and .42. Coefficients of variation in group I were the lowest for the three respective normalization methods (.33, .36, and .33), whereas those in group III were the highest (.75, .61, and .54). Group II had intermediate variability, with respective coefficients of variation of .43, .61, and .37.

Analysis of membrane yield and receptor functionality in membrane preparations derived from nonfailing and failing human ventricular myocardium. The amount of tissue removed at the time of endomyocardial biopsy was not sufficient to perform membrane marker measurements, leading us to assess membrane marker behavior in larger right ventricular endomyocardial samples removed from explanted human hearts (see Assay char-

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acterization). To investigate the possibility that differential yield of functioning membrane could account for a lower β-receptor density in failing compared with nonfailing human ventricular myocardium and to assess the functional consequences of β-receptor down-regulation, we compared membrane marker activity and isoproterenol stimulation of adenylate cyclase in membrane derived from right and left ventricular free wall samples prepared identically to endomyocardial biopsy tissue. Four nonfailing left and right ventricles were taken from prospective donors aged 19 to 27 years whose hearts were not used for transplantation because of no suitable recipient match (n = 3) or atrioventricular valve regurgitation with normal systolic function (n = 1). Failing left and right ventricles were taken from eight recipients with severe biventricular failure and hemodynamic data similar to those found in group III (table 3): mean right atrial pressure, 13.7 ± 2.7 mm Hg; mean pulmonary wedge pressure, 26.8 ± 3.4 mm Hg; cardiac index, 2.1 ± 0.2 liters/min/m²; and ejection fraction, 0.15 ± 0.03. Age and sex distributions were also similar to those of group III.

As shown in table 5, compared with four nonfailing left and right ventricles, eight failing left and right ventricles had similar values for the membrane markers basal adenylate cyclase activity, fluoride- and forskolin-stimulated adenylate cyclase activities, and 5' nucleotidase activity. However, the failing ventricles had a significantly reduced β-receptor density and significantly lower net isoproterenol-stimulated adenylate

![FIGURE 4](image_url)

**TABLE 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>No. of men</th>
<th>NYHA class</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n = 7)</td>
<td>42.2</td>
<td>3</td>
<td>1.0</td>
</tr>
<tr>
<td>± 2.8</td>
<td>± 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II (n = 10)</td>
<td>45.9</td>
<td>8</td>
<td>2.3</td>
</tr>
<tr>
<td>± 4.5</td>
<td>± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III (n = 19)</td>
<td>37.9</td>
<td>17</td>
<td>3.4 ± 0.6</td>
</tr>
<tr>
<td>± 2.5</td>
<td>± 0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < .05 vs I; **p < .05 vs II.*

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean right atrial pressure (mm Hg)</th>
<th>Mean pulmonary wedge pressure (mm Hg)</th>
<th>Cardiac index (l/min/m²)</th>
<th>LVEF</th>
</tr>
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<tbody>
<tr>
<td>I (n = 7)</td>
<td>2.4</td>
<td>6.3</td>
<td>3.3</td>
<td>0.59</td>
</tr>
<tr>
<td>± 0.4</td>
<td>± 1.1</td>
<td>± 0.4</td>
<td>± 0.01</td>
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<tr>
<td>II (n = 10)</td>
<td>5.8</td>
<td>12.4</td>
<td>2.4</td>
<td>0.38</td>
</tr>
<tr>
<td>± 1.4</td>
<td>± 2.6</td>
<td>± 0.1</td>
<td>± 0.02</td>
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<tr>
<td>III (n = 19)</td>
<td>11.2 ± 0.6</td>
<td>25.0 ± 0.6</td>
<td>2.1 ± 0.1</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>± 1.5</td>
<td>± 1.8</td>
<td>± 0.2</td>
<td>± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

*p < .05 vs I; **p < .05 vs II.*
PATHOPHYSIOLOGY AND NATURAL HISTORY—VENTRICAL PERFORMANCE

Table 4
Receptor binding data (mean ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Bmax, fmol per:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg protein (Lowry)</td>
<td>mg protein (Bradford)</td>
</tr>
<tr>
<td>I (n = 7)</td>
<td>106.5 ± 13.1</td>
<td>147.0 ± 20.2</td>
</tr>
<tr>
<td>II (n = 10)</td>
<td>47.6 ± 6.1</td>
<td>91.7 ± 20.9</td>
</tr>
<tr>
<td>III (n = 19)</td>
<td>35.4 ± 6.7</td>
<td>69.0 ± 11.0</td>
</tr>
</tbody>
</table>

*a p < .05 vs I; b p < .05 vs II.

cyclase activity and a lower isoproterenol/fluoride ratio.

Morphologic analysis. With the exception of three subjects with doxorubicin cardiomyopathy, biopsy specimens from all subjects in groups II and III demonstrated varying degrees of hypertrophy on light microscopy. Four subjects in group II and 13 subjects in group III had evidence for increased interstitial fibrosis. Only one specimen in group I had evidence for increased fibrosis and hypertrophy. Of the six subjects in group I who were evaluated for antracycline changes, the mean (± SD) score on the Billingham scale was 1.42 ± 0.49 (mild amount of damage, < 15% cells involved). Of the three subjects in groups II and III who received doxorubicin, two subjects in group II had respective biopsy scores of grade 1 and 2.5 and were thought to have “mixed etiology” cardiomyopathy with doxorubicin as a contributing factor, whereas the group III subject had a grade 3.0 biopsy score and was diagnosed as having uncomplicated antracycline cardiomyopathy.

In group II there was no significant difference in receptor density in biopsy specimens without and with increased interstitial fibrosis (with, Bmax/mg Lowry protein = 43.8 ± 12.7 fmol/mg, Bmax/gram wet weight = 1146 fmol/g; without, Bmax/Lowry protein = 51.0 ± 8.5, Bmax/gram wet weight = 1416 fmol/g, both p = NS). In group III there was also no significant difference (p > .05) in β-receptor density in biopsy samples with and without fibrosis, although those biopsies with fibrosis tended to have a lower receptor density when normalized to the Lowry protein method (with, Bmax/mg Lowry protein = 27.1 ± 6.4 fmol/mg, Bmax/gram wet weight = 780 fmol/g; without, Bmax/mg Lowry protein = 55.6 ± 14.2 fmol/mg, Bmax/gram wet weight = 963 fmol/g).

Table 5
Comparison of membrane markers and β-adrenergic receptor indexes in crude membrane prepared from right and left ventricular free wall in four nonfailing and eight failing hearts (mean ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>ICVP</th>
<th>Bmax (fmol/mg)</th>
<th>Basal activity</th>
<th>Net stimulation</th>
<th>5'Nucleotidase activity (nmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adenylate cyclase (pmol/min/mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Isoproterenol</td>
<td>Fluoride</td>
<td>Forskolin</td>
</tr>
<tr>
<td>NF</td>
<td></td>
<td></td>
<td>14.1</td>
<td>34.1</td>
<td>43.7</td>
</tr>
<tr>
<td>LV</td>
<td>71.1</td>
<td>± 11.7</td>
<td>± 1.6</td>
<td>± 3.5</td>
<td>± 3.6</td>
</tr>
<tr>
<td>RV</td>
<td>67.0</td>
<td>± 12.8</td>
<td>± 2.5</td>
<td>± 6.5</td>
<td>± 10.4</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
<td>10.7</td>
<td>15.3</td>
<td>47.2</td>
</tr>
<tr>
<td>LV</td>
<td>41.0A</td>
<td>± 8.3</td>
<td>± 1.8</td>
<td>± 1.8</td>
<td>± 8.4</td>
</tr>
<tr>
<td>RV</td>
<td>28.8A</td>
<td>± 3.9</td>
<td>± 1.2</td>
<td>± 1.6</td>
<td>± 5.4</td>
</tr>
</tbody>
</table>

NF = nonfailing; F = failing; other abbreviations as in table 1.

a p < .05 vs respective NF group; b p < .10 vs respective NF group.
The positive inotropic response to dobutamine, reflect-
ed by the increase in left ventricular stroke work index
(table 7) and peak positive left ventricular dP/dt (table
7, figure 6), was greater in group A than group B (p <
.05) despite similar effects on left ventricular end-
diastolic pressure in group A (table 7). In contrast,
group A and B subjects did not differ in their response
in peak left ventricular dP/dt or stroke work index
during infusion of calcium gluconate (figure 6, table
7). Modest increases in peak positive dP/dt, cardiac
index, and stroke work index were accompanied by a
call in left ventricular end-diastolic pressure and an
increase in mean arterial pressures (table 7, figure 6) in
both groups receiving calcium gluconate.

Discussion

Studies of \( \beta \)-adrenergic receptors in human hearts
have been limited by the gram quantities of tissue
required for receptor identification by radioligand
binding methods. Using the high–specific activity,
high-specificity radioligand ICYP, we have success-
fully identified \( \beta \)-adrenergic receptors in milligram
quantities of human endomyocardial biopsy tissue.
The results of ICYP binding in crude membranes de-

derived from right ventricular endomyocardium indicate
that this radioligand fulfills the criteria for specific
binding to \( \beta \)-adrenergic receptors. ICYP binding was

saturable, stereospecific, and linear with respect to
varying protein concentrations. The rank-order of po-
tency for \( \beta \)-agonists was that of a predominantly \( \beta_1 \)

population, with norepinephrine having similar affini-
ty to epinephrine. These findings and the results of
kinetic data do not differ substantially from previous
results in membranes derived from guinea pig ventric-

ular myocardium,\(^\text{11}\) allowing for differences in tem-
perature at which the experiments were performed.
Scatchard analysis indicated that ICYP recognizes the
endomyocardial \( \beta \)-receptor population as a single class
of binding sites within the range of radioligand con-
centrations examined.

We employed a modification of a previously de-
scribed high-yield preparation of crude myocardial
membranes that has a yield of 90% and an enrichment
of \( \beta \)-adrenergic receptors of twofold to threefold rela-
tive to the washed and pelleted homogenate.\(^\text{21}\) Al-
though the amount of material removed by endomyo-

cardial biopsy was not sufficient for performance of
sarcolemmal marker enzyme measurements, we per-
formed such measurements in small aliquots of right
ventricular septal endomyocardium taken from trans-
plant donor or recipient hearts. Data from right ventric-
ular endomyocardium indicated that relative to left and

---

TABLE 6
Baseline hemodynamic values and \( \beta \)-adrenergic receptor density in
subgroups A and B (unpaced, mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Subgroup A</th>
<th>Subgroup B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(LVEF &gt;0.40) p value</td>
<td>(LVEF &lt;0.30)</td>
</tr>
<tr>
<td>LVEF</td>
<td>0.43 ± 0.01</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>( \beta )-Adrenergic receptor density (fmol/g wet wt)</td>
<td>1568 ± 375 &lt; .005</td>
<td>792 ± 335</td>
</tr>
<tr>
<td>( K_d ) (pM)</td>
<td>27.0 ± 9.5 NS</td>
<td>27.9 ± 7.0 NS</td>
</tr>
<tr>
<td>Cardiac index</td>
<td>2.67 ± 0.54 NS</td>
<td>2.36 ± 0.19 NS</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>103 ± 8 &lt; .05</td>
<td>75 ± 3</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mm Hg)</td>
<td>10 ± 4 &lt; .001</td>
<td>28 ± 1</td>
</tr>
<tr>
<td>Right atrial pressure (mm Hg)</td>
<td>8 ± 1 NS</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>91 ± 9 NS</td>
<td>92 ± 4</td>
</tr>
<tr>
<td>Peak left ventricular dP/dt (mm Hg/sec)</td>
<td>993 ± 105 NS</td>
<td>812 ± 51</td>
</tr>
<tr>
<td>Stroke work index (g·m/kg)</td>
<td>39.9 ± 4.7 &lt; .005</td>
<td>16.2 ± 1.4</td>
</tr>
</tbody>
</table>

---

FIGURE 5. Serum ionized calcium levels in groups A and B at baseline and during the 2.5, 5, and 10 mg/kg/min infusions of calcium gluconate.
right ventricular free wall, membranes prepared from endomyocardial tissue have comparable numbers of β-adrenergic receptors and 5’ nucleotidase activity but lower enzyme activity for adenylate cyclase. Because all types of adenylate cyclase activity (basal and all forms of stimulation) were lower in endomyocardial tissue, it is possible that, compared with full-thickness right ventricular free wall, human right ventricular endomyocardium contains less cyclase catalytic subunit per amount of β-adrenergic receptors or amount of functioning membrane.

Morphologic analysis of tissue revealed the expected findings of hypertrophy in groups II and III, as well as mild anthracycline changes in the six subjects treated with anthracycline antibiotics in group I. In the subjects in groups II and III who had increased interstitial fibrosis, there was no significant relationship between the presence of fibrosis and decreased β-receptor density, perhaps in part because the Lowry method of protein measurement does not recognize collagen. 18 The group I mean B<sub>max</sub> values were not significantly different from data derived from gram quantities of normally-functioning right ventricular myocardium assessed by ICYP binding, and previous analysis of β-receptor density in membranes derived from hypertrophic human heart has revealed no difference in membrane markers as compared with normal 21 as well as good agreement in receptor density normalized by sev-

### TABLE 7
Absolute values during infusion of dobutamine and calcium in subgroups A and B (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Dobutamine (µg/kg/min)</th>
<th>Calcium gluconate (mg/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>2.1</td>
</tr>
<tr>
<td>Peak left ventricular dP/dt (mm Hg/sec)</td>
<td></td>
<td>1056 ± 127</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>849 ± 41</td>
<td>996 ± 60</td>
</tr>
<tr>
<td>Stroke work index (g-m/m²)</td>
<td></td>
<td>31 ± 3</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac index (l/min/m²)</td>
<td></td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td></td>
<td>102 ± 6</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>77 ± 2</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mm Hg)</td>
<td></td>
<td>13 ± 2</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Statistical difference in the net increase from baseline between the two groups.
eral different methods, including normalization to method of contractile protein determination. Therefore the relatively mild structural abnormalities found in some biopsy specimens did not appear to affect receptor density. This is in agreement with a more detailed analysis of 27 failing left ventricles from transplant recipients, in which no correlation was found between β-receptor density and semiquantitative scores of hypertrophy (r = .02, p = NS) and fibrosis (r = .15, p = NS), unpublished data).

Although the normal group (group I) contained a significantly higher percentage of women as compared with the severely failing group (group III), the values in men and women were identical in group I, and we have not found any gender difference in β-adrenergic receptor density in previous studies in normal or failing human hearts. Therefore, there does not appear to be any apparent explanation for the reduction in β-adrenergic receptor density in groups II and III other than heart failure.

The downward trend of β-receptor density in the three groups as biventricular dysfunction increased indicated a significant relationship between decrease in β-receptor density and the degree of heart failure. The fact that ICYP dissociation constants were not different among the three groups supports the concept that myocardial β-adrenergic receptors are not structurally altered by heart failure, and that the decrease in Bmax is secondary to a decrease in receptor density. This down-regulation of the human ventricular β-adrenergic receptor in heart failure is quantitatively similar to previously published findings in gram quantities of human heart, in which severely failing left ventricles were found to have reductions in receptor density of 50% to 53%, similar to the reduction of 53% to 67% in severely failing right ventricles in the current study.

The ability to obtain cardiac tissue from ambulatory subjects by catheterization procedures allowed us to assess for the first time myocardial β-receptor density in subjects with mild to moderate heart failure. The data indicated that β-receptor density is decreased in this group as compared with normals but that receptor density is higher than that encountered in the more severely failing group. The collective statistical analysis of these data was consistent with a stepwise reduction in cardiac β-receptor density progressing from normal function to mild-to-moderate dysfunction to severe dysfunction. Therefore, β-receptor down-regulation in the failing human heart appears to occur even in subjects with mild-to-moderate heart failure and is not strictly confined to subjects with severe failure.

The pharmacologic consequences of a reduction in β-receptor density in the intact human heart were further examined in a subgroup of seven minimally to mildly failing and 11 severely failing hearts that were given dobutamine and calcium sequentially. In the severely failing subgroup, the LVEF and β-receptor density were lower by 58% and 50%. Infusion of dobutamine, a β-agonist that is only minimally selective for β1-receptors, resulted in a marked blunting of the left ventricular (dP/dt) contractility response in the severely failing group. In contrast, the contractile response to calcium, an agent that acts beyond the β-adrenergic receptor, was not different in the mildly and severely failing groups. Moreover, quantitative aspects of the β-agonist–mediated decrease in contractility in the severely failing group (decrease in dP/dt by 59% at the highest dose of dobutamine administered) were quite similar to previous results in isolated human tissue in which receptor density differed by 50%. These data therefore confirm that the reductions in β-adrenergic receptor density and receptor pathway responsiveness previously found in explanted severely failing human hearts are also present in the intact heart.

Peak positive left ventricular dP/dt was used to evaluate contractile response to dobutamine and calcium in the two groups of patients because the β-adrenergic receptor is coupled to a positive inotropic response and peak left ventricular dP/dt is easily measured. Although peak positive left ventricular dP/dt is dependent on loading conditions and heart rate, in our patients the changes in left ventricular end-diastolic pressure were similar in the two groups of patients, and any increase in heart rate was minimized by making all measurements after 2 min of atrial pacing at 110 or 115 beats/min. Because mean arterial pressure rose slightly more with dobutamine in group B patients, which would tend to increase dP/dt, this does not account for the reduced inotropic response in these subjects. Assessments of inotropic responses to dobutamine and calcium by load-independent indexes of contractility, such as the slope of the end-systolic pressure-volume relationship at different afterloads, might have overcome any hidden bias introduced by different effects on preload or afterload in the two patient groups. However, this type of study would have been very difficult to perform repeatedly during incremental infusions of two drugs in this patient population.

Because of limitations in the amount of tissue that can be removed by endomyocardial biopsy, other potentially important biochemical variables such as agonist affinity, adenylate cyclase responsiveness, and β1/β2 subtyping could not be measured in this study. However, all these measurements have been previous-
ly performed in gram-quantity aliquots of explanted human heart, in which no apparent significant change in $\beta$-agonist affinity occurs,$^{25}$ adenylate cyclase responsiveness is blunted,$^{1,4}$ and $\beta_2$-receptors down-regulate to a much greater extent than $\beta_2$-receptors.$^{21}$ Based on the amount of tissue required to perform each of these determinations, in endomyocardial biopsy tissue only one of these assays or a receptor density measurement can be performed at any one time. Since any of the four can be performed in endomyocardial biopsy tissue by means of modifications of the methods outlined in this investigation, ultimately all these aspects of $\beta$-adrenergic receptor regulation can be examined in the intact human heart.

Mechanisms responsible for $\beta$-adrenergic receptor down-regulation in the failing human heart have not been addressed by this study. However, in an ongoing investigation (Bristow MR, unpublished data) testing the hypothesis that adrenergic stimulation accounts for $\beta$-receptor down-regulation in diffuse cardiomyopathy ($n = 15$), cardiac venous norepinephrine level (mean $\pm$ SEM, $609 \pm 156$ pg/ml) and coronary sinus norepinephrine level ($740 \pm 166$ pg/ml) correlate inversely and significantly ($r = .73$ for central venous, $.66$ for coronary sinus, both $p < .05$) with $\beta$-adrenergic receptor density in right ventricular biopsy specimens ($31.0 \pm 5.3$ fmol/mg). Thus exposure to endogenous $\beta$-agonists could account for myocardial $\beta$-adrenergic down-regulation in the failing human heart.$^{26}$ However, other explanations for heart failure--associated $\beta$-adrenergic receptor down-regulation are also possible.

Observations made in this investigation provide additional evidence for the potential importance of myocardial $\beta$-adrenergic receptor regulatory events in the natural history of heart failure. The decreased $\beta$-receptor density would be expected to compromise the ability of endogenous catecholamines to support cardiac function,$^{1,4,7}$ and this effect may be manifest relatively early in the clinical course at a time when only mild to moderate heart failure is present.

Right ventricular endomyocardial biopsy is currently a readily available technique in many medical centers and has been shown to be as safe as other cardiac catheterization procedures.$^{8-10}$ The ability to measure $\beta$-adrenergic receptors in intact human hearts can potentially increase our understanding of the myocardial cellular changes that occur in heart failure and of changes produced by drug therapy. With the techniques described in this study, hypotheses concerning $\beta$-adrenergic receptor regulation in the human heart can be easily tested prospectively in intact human subjects.

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M B Fowler, J A Laser, G L Hopkins, W Minobe and M R Bristow

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