Heterogeneous Transmural Distribution of β-Adrenergic Receptor Subtypes in Failing Human Hearts

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Background. Downregulation of myocardial β-adrenergic receptor density does not occur in a spatially uniform distribution in patients with congestive heart failure. Rather, it results primarily from loss of receptors in the subendocardium. In patients with dilated cardiomyopathy, β₁-receptors have been found to be downregulated selectively. These observations suggest that considerable transmural heterogeneity in the distribution of β-adrenergic receptor subtypes exists in the failing human heart. The present study was designed to test this hypothesis.

Methods and Results. We used quantitative autoradiography of radioligand binding sites to measure the distribution of β-adrenergic receptor subtypes in transmural sections of left ventricular myocardium obtained from cardiac transplant patients with ischemic (n=13) and idiopathic dilated (n=12) cardiomyopathy and from 4 subjects with no history of congestive heart failure. Analysis of radioligand binding isotherms revealed a significant reduction in total β-adrenergic receptor density in hearts of patients with ischemic and idiopathic cardiomyopathy (20.2±1.9 and 18.2±2.0 fmol/mg protein, respectively, versus 40.0±11.4 in control subjects; P<.01 for both). Loss of the β₁-subtype accounted for 86% of the total reduction in β-receptor density in failing hearts. Despite the significant decreases in overall tissue receptor content, the densities of total β-receptors and β-receptor subtypes in subepicardial myocytes were equivalent in failing and control hearts. However, in contrast to control hearts, in which the transmural distribution of total and β₁-receptors was uniform (endocardial:epicardial receptor density ratios, 0.97±0.14 and 1.0±0.2, respectively), hearts of patients with ischemic and idiopathic dilated cardiomyopathy had significantly lower total β-receptor and β₁-receptor densities in the subendocardium (ratios, 0.66±0.06 and 0.46±0.09 for total and β₁-receptors, respectively, in ischemic cardiomyopathy and 0.60±0.08 and 0.52±0.11 in dilated cardiomyopathy; P<.001 for all values compared with a ratio of 1).

Thus, β₁:β₂ receptor density ratios were markedly decreased in the subendocardium of ischemic and idiopathic dilated left ventricles compared with control hearts.

Conclusions. A significant transmural gradient in the density of myocardial β₁-adrenergic receptors exists in the hearts of patients with ischemic and dilated cardiomyopathy, resulting in a markedly altered β₁:β₂ receptor density ratio in the subendocardium. (Circulation. 1993;88:2501-2509.)

Key Words • cardiomyopathy • radiography • radioligand binding

Myocardial β-adrenergic receptor density and responsiveness are known to be diminished in patients with congestive heart failure, but the mechanisms underlying receptor downregulation are incompletely understood. Local factors, rather than systemic conditions, appear to play a principal role. Considerable spatial heterogeneity in β-adrenergic receptor subtype downregulation may occur and could contribute to electrophysiological heterogeneity, leading to malignant ventricular tachyarrhythmias.

Although Bristow et al have analyzed the distribution of β-adrenergic receptors in multiple membrane fractions of myocardium from patients with pulmonary hypertension, most previous studies of altered β-adrenergic receptor density and function in diseased myocardium have used conventional membrane fractions (30 000 to 50 000g pellets) prepared from homogenates of whole tissue, an approach with limited anatomic resolution that also entails other unavoidable shortcomings. Crude membrane preparations comprise mixtures of cell surface and intracellular membranes of multiple cell types. In addition, conventional membrane preparations (30 000 to 50 000g pellets) are typically depleted of the putative intracellular compartment (light vesicle fraction) in which β-receptors are thought to be sequestered in response to prolonged agonist stimulation. To overcome these limitations, we have developed quantitative autoradiographic techniques to characterize the total tissue content of β-receptors in transmural sections of the heart. Using this approach, we observed that downregulation of β-adrenergic receptors in failing human myocardium did not occur uniformly in the transmural distribution. Rather, decreased β-receptor density was found to be a result of a selective reduction of β-receptors on subendocardial myocytes.
TABLE 1. Clinical Features of Cardiac Transplant Recipients

| Age, y | 47.8±2.6 |
| Race  |         |
| White | 22       |
| Black | 3        |
| Sex   |         |
| Male  | 23       |
| Female| 2        |
| NYHA functional class |         |
| II    | 2        |
| III   | 8        |
| IV    | 15       |
| Hemodynamic Index |         |
| Cardiac output, L/min | 4.7±0.3 |
| Cardiac index, L·min⁻¹·m⁻² | 2.4±0.1 |
| LVEF, % | 22.3±1.6 |
| PCWP, mm Hg | 19.3±1.4 |

NYHA indicates New York Heart Association; LVEF, left ventricular ejection fraction; and PCWP, pulmonary capillary wedge pressure.

Bristow et al² have shown that β-adrenergic receptor downregulation in patients with idiopathic dilated cardiomyopathy is attributable mainly to reduction of the β₁-adrenergic receptor subtype. This important observation, coupled with our findings of a nonuniform distribution of β-receptors in the failing heart, suggests that a substantial transmural gradient might exist in the distribution of β₁-adrenergic receptor subtypes in failing human myocardium. Accordingly, the goal of the present study was to quantitatively delineate the transmural distribution of myocardial β₁-adrenergic receptor subtypes and determine whether regional heterogeneity occurs in the distribution of myocardial β₁-receptor subtypes in patients with chronic congestive heart failure.

Methods

Tissue Procurement

Transmural samples of left ventricular myocardium were obtained from hearts of 13 patients with ischemic cardiomyopathy and 12 patients with idiopathic dilated cardiomyopathy who underwent cardiac transplantation at Barnes Hospital from March 1991 to October 1992. Selected clinical features of these transplant recipients are shown in Table 1. Eleven patients were being treated with intravenous dobutamine (mean dose, 7.0±1.2 μg·kg⁻¹·min⁻¹) at the time of transplantation, and all patients were taking other medications, including diuretics, digoxin, and angiotensin-converting enzyme inhibitors or other vasodilators.

Transmural samples of left ventricular myocardium were also obtained from four nonfailing control hearts. These samples came from three vital organ donors and one valve homograft donor whose hearts were not used for transplantation. These donors included two motor vehicle accident victims (a 38-year-old man and 3-year-old boy) with massive closed-head injury, a 43-year-old woman with brainstem herniation following cerebrovascular accident, and a 27-year-old man who died of a gunshot wound to the head. All four subjects had been treated with intravenous dopamine (mean dose, 8.4±2.6 μg·kg⁻¹·min⁻¹) and one patient had received intravenous dobutamine (3.6 μg·kg⁻¹·min⁻¹) within 24 hours of tissue harvest, but none had a clinical history of congestive heart failure. Informed consent was obtained for use of organs in research in all cases. The study protocol was approved by the Washington University Human Studies Committee.

Excised hearts were rinsed immediately in cold modified Krebs solution (containing in mmol/L: NaCl 118, KCl 4.7, CaCl₂ 2.5, MgCl₂ 0.54, NaHCO₃ 25, NaH₂PO₄ 1, dextrose 11, ascorbic acid 0.001, pargyline 0.102, and EDTA 0.050), and transmural blocks of ventricular myocardium were dissected, frozen, and stored in sealed containers at −70°C until used in experiments. Regions of grossly discernible myocardial necrosis or fibrosis were excluded.

Radioiodin Binding Assays

The total tissue content of β₁-adrenergic receptors in transmural sections of left ventricular myocardium was determined with radioligand binding isotherms. Transmural, 12-μm-thick frozen sections of unfixed tissue were cut with a cryostat-microtome and mounted on gelatin-coated slides. Slide-mounted tissue sections were incubated at 37°C in buffer (NaCl 154 mmol/L, MgCl₂ 10 mmol/L, Tris-HCl 10 mmol/L, pH 7.4) containing selected concentrations (1.1 to 107 pmol/L) of (−)-[¹²⁵]iodocyanopindolol (ICYP) (1900 to 2200 Ci/mmol; New England Nuclear, Boston, Mass). Nonspecific binding was defined as binding of radioligand in the presence of 10⁻⁶ mol/L l-propranolol. Sections were incubated with radioligand under conditions found in previous studies to establish steady-state binding. Large volumes of buffer (50 mL) were used so that the concentration of free radioligand did not change measurably during incubation intervals. After selected intervals of radioligand binding, sections were rinsed for 1 hour at 22°C in buffer that did not contain radioligand or unlabeled displacer, conditions previously shown to selectively remove nonspecifically bound radioactivity without removing specifically bound radioactivity. Sections were then dipped briefly in distilled water to remove buffer solutes and air-dried. The amount of radioligand bound in each section was determined by scraping sections from the slides with a razor and quantifying radioactivity in each with gamma scintillation spectrometry. In all experiments, data points were calculated as means of duplicate or triplicate determinations. Specifically bound radioactivity was normalized to account for modest variations in section thickness, section size, or amounts of interstitial fibrosis by measuring the total area and protein content in sets of transmural sections obtained at regular intervals during the preparation of large numbers of serial sections during each individual experiment. Areas were measured by digitizing the outlines of enlarged photographs of the sections with computer-assisted planimetry. Cellular protein was assessed in individual sections scraped from non-gelatin-coated slides by the method of Lowry.
et al. using bovine serum albumin standards. Because this method is insensitive to collagen and other extracellular matrix proteins, effects on apparent receptor density caused by potential differences in the relative amounts of interstitial fibrosis that occur among failing and nonfailing ventricles were minimized.

The relative proportions of $\beta_1$- and $\beta_2$-receptors in left ventricular myocardium were determined by incubating transmural sections with ICYP in the presence of CGP-20712A, a highly selective $\beta_1$-antagonist, under defined conditions in which the unlabeled displacer inhibited radioligand binding to $\beta_2$-receptors by nearly 100% without significantly affecting binding to $\beta_2$-receptors. Optimal conditions for this purpose were determined in preliminary competition binding experiments in which transmural sections from two nonfailing and four failing hearts were incubated under equilibrium binding conditions with 25 pmol/L ICYP and 18 separate concentrations of CGP-20712A ranging from $1 \times 10^{-6}$ mol/L to $3 \times 10^{-5}$ mol/L. Total $\beta$-receptor binding in each experiment was determined in additional sections incubated with 25 pmol/L ICYP with or without $10^{-4}$ mol/L 1-propranolol. After incubation for 1 hour at 37°C, sections were rinsed, dried, and scraped, and radioactivity was quantified as described for binding isotherms. With analysis of the competition binding curves (as described below and as shown in the "Results" section), we identified a specific concentration of CGP-20712A, $1 \times 10^{-5}$ mol/L, that maximally inhibited ICYP binding to $\beta_1$-receptors while minimally affecting binding to $\beta_2$-receptors. Therefore, in subsequent experiments, transmural sections of failing and nonfailing left ventricle were incubated with 25 pmol/L ICYP and $10^{-7}$ mol/L CGP-20712A. The proportions of receptor subtypes were determined by quantifying radioactivity in scraped sections or by performing quantitative autoradiographic studies as described below.

### Quantitative Autoradiography

 Autoradiographic methods have been extensively validated in previous studies. Briefly, gelatin-coated coverslips were coated with Kodak NTB2 nuclear track emulsion (Eastman Kodak, Rochester, NY), dried at room temperature for at least 3 hours, and glued at one end to slides containing radiolabeled sections. After the emulsion was exposed for approximately 24 hours, the unglued edge of each coverslip was gently lifted from the slides, and the emulsion was developed with Kodak D19 developer (diluted 1:1 with water) for 3 minutes and fixed with Kodak fixer for 1 minute at 25°C. The tissue sections were then stained with hematoxylin and eosin, and the coverslips were sealed permanently to the slides.

The tissue and overlying developing grains in the emulsion layer were examined by light microscopy. The densities of total $\beta$-adrenergic receptors and the proportions of $\beta_1$- and $\beta_2$-receptors in cardiac myocytes of the subepicardium and the subendocardium (defined as the region of tissue contained within five high-power fields of the epicardial and endocardial surfaces, respectively, at a magnification of $630 \times$) were determined by counting grains. Grain densities were compared in corresponding regions of serial sections incubated with 25 pmol/L ICYP with or without 1-propranolol (the sum of $\beta_1$- and $\beta_2$-receptors) and those incubated with 25 pmol/L ICYP and $10^{-7}$ mol/L of CGP-20712A ($\beta_2$-receptors only). The grain density values were corrected to account for the modest amount of ICYP binding to $\beta_1$-receptors and the modest inhibition of ICYP binding to $\beta_2$-receptors under the conditions used (see below).

Data from individual sections were determined by calculating means of grain density measurements from at least 10 distinct microscopic fields. Composite data points were derived from the average of at least three separate sets of transmural sections per patient.

### Data Analysis

 Competition binding curves were analyzed with the iterative curve-fitting program (LIGAND) of Munson and Rodbard as modified for microcomputers by McPherson. With this method, initial estimates of selected binding constants in the defined model are iteratively refined by nonlinear least-squares curve-fitting techniques based on the Marquardt-Levenberg modification of the Gauss-Newton method. When the weighted sum of the squares was minimized, final parameter estimates were generated and fitted to the actual data.

 The extent to which selected concentrations of CGP-20712A inhibited binding of ICYP to $\beta_1$- and $\beta_2$-receptors was determined with the following equation of Neve et al. describing the inhibition of a selective radioligand by a selective competing ligand:

$$B = \frac{B_{max \cdot L}}{L + K_{d1} \cdot (1 + i/K_{i1})} + \frac{B_{max \cdot L}}{L + K_{d2} \cdot (1 + i/K_{i2})}$$

where $B$ is the amount of radioligand bound; $B_{max}$ and $B_{max}$ are the densities of $\beta_1$- and $\beta_2$-receptors; $L$ is the concentration of radioligand; $i$ is the concentration of unlabeled displacer; $K_{d1}$ and $K_{d2}$ are dissociation constants of $\beta_1$- and $\beta_2$-receptors, respectively, for the radioligand; and $K_{i1}$ and $K_{i2}$ are dissociation constants of the receptors for the unlabeled competing ligands.

We measured $K_d$ by Scatchard transformation of binding isotherm data and, because $\beta_1$-receptors make up approximately 65% to 75% of myocardial $\beta$-receptors, we designated this observed value as $K_{d1}$. Because ICYP is approximately twofold selective for $\beta_2$-receptors, $K_{d2}$ was assumed to be equal to 50% of $K_{d1}$. Values for $K_{d1}$ and $K_{d2}$ were determined by analysis of detailed competition binding curves as described above and as shown in Table 2. Using these values and the values for the concentrations of radioligand (L) and displacing ligand (i) and specific binding (B), the relative extent to which CGP-20712A inhibited ICYP binding to $\beta_1$- and $\beta_2$-receptors was calculated.

All data are expressed as mean ± SEM. Statistical analyses were performed on a Macintosh IIci computer using the STATVIEW 4.0 application package. Two-sided unpaired $t$ tests were used to determine statistically
significant differences in binding isotherm and grain density measurements. Ratios of total β-receptor and β-receptor subtype densities were analyzed with one-sample t tests by comparing the observed means to a hypothesized mean of one. Significance was defined as 

\[ P < 0.05 \] in all cases.

Results

**CGP-20712A Competition Binding Studies**

Composite competition binding curves determined in transmural left ventricular sections prepared from two nonfailing and four failing hearts are shown in Fig 1. The data were best fit by biphasic curves indicating the presence of two binding sites. Analysis with the LIGAND program indicated that the affinities of the two binding sites for CGP-20712A differed by approximately three orders of magnitude (see Table 2). Using Equation 1, we determined that at a concentration of \( 10^{-7} \) mol/L, CGP-20712A inhibited binding of 25 pmol/L ICYP to β₁ sites by 95.5% and 95.1% in control and failing hearts, respectively, whereas binding to β₂ sites was inhibited by only 0.5% and 1.6%, respectively. Thus, all subsequent studies designed to determine the relative densities of β-receptor subtypes were performed by incubating sections with 25 pmol/L ICYP and \( 10^{-7} \) mol/L CGP-20712A. All radioactivity and grain density measurements were corrected to account for the 4% to 5% binding of ICYP to β₁-receptors and the 1% to 2% inhibition of radioligand binding to β₂-receptors.

**Radioligand Binding Isotherms**

The total content of β-adrenergic receptors in transmural sections was determined by Scatchard analysis of binding isotherms. Representative isotherms and Scatchard plots are shown in Fig 2, and composite results in failing and control left ventricles are shown in Fig 3. The total tissue content of β₂-receptors (\( B_{\text{max}} \)) in the four nonfailing ventricles was 40.0 ± 11.4 fmol/mg protein. Total β₂-receptor density was reduced significantly in the
combined group of all failing ventricles (B_{max}=19.3±1.4 fmol/mg protein, P<.001) and in subgroups of failing ventricles divided according to pathogenesis (B_{max}=18.2±2.0, P<.01 and B_{max}=20.3±1.9, P=.01 in idiopathic dilated and ischemic cardiomyopathy, respectively). Treatment with intravenous dobutamine before transplantation had no apparent effect on the total tissue content of β-receptors (B_{max}=19.1±2.6 fmol/mg protein in patients treated with dobutamine versus 19.4±1.5 in patients not treated with dobutamine, P=NS).

The reduction in β-receptor density observed in failing ventricles was attributable primarily to a loss of the β_{1}-receptor subtype (Fig 3). The β_{1}-receptor density decreased from 30.2 fmol/mg protein in nonfailing ventricles to 12.4 fmol/mg protein in failing ventricles. Thus, 86% of the total reduction in β-receptor density in failing hearts was attributable to this 17.8 fmol/mg protein decrease in β_{1}-subtype density. The β_{1}-receptor densities were not significantly different in left ventricles from patients with idiopathic dilated (11.6 fmol/mg protein) and ischemic (13.1 fmol/mg protein) cardiomyopathy. No significant differences were observed in β_{2}-subtype density (Fig 3) in transmural slices of nonfailing (9.8 fmol/mg protein) and failing (6.9 fmol/mg protein) hearts, nor were any differences in β_{2}-receptor density noted between heart failure patients with idiopathic (6.6 fmol/mg protein) or ischemic (7.2 fmol/mg protein) cardiomyopathy.

** Autoradiographic Analyses 

The distribution of β-adrenergic receptors in left ventricular subepicardial and subendocardial regions of compact myocytes was determined by autoradiographic analysis of transmural sections prepared from 4 nonfailing and 23 failing left ventricles. Representative autoradiographs are shown in Fig 4.

Despite the significant reduction of total β-receptor density (attributable almost entirely to reduced β_{1}-receptor density) in whole transmural slices of failing ventricles compared with nonfailing controls, no significant differences were observed in the density of total β-receptors or β-receptor subtypes in subepicardial myocytes regions of control and failing hearts (Fig 5). In marked contrast, however, total β-receptor density and β_{1}-receptor density were reduced significantly in subendocardial myocytes in the combined group of all failing hearts and in both subgroups of patients with idiopathic dilated and ischemic cardiomyopathies (Fig 5).

The density of total β-receptors was not significantly different in subendocardial and subepicardial myocyte regions of nonfailing ventricles (endoocardial:epicardial receptor density ratio, 0.97±0.14) (Fig 6), whereas in failing ventricles, total β-receptor density was significantly lower in subendocardial myocytes than in subepicardial myocytes (ratio, 0.63±0.05, P<.0001). The endocardial:epicardial total β-receptor density ratio was similar in patients with idiopathic and ischemic cardiomyopathy (Fig 6). Moreover, as shown in Fig 7, the distribution of β_{1}-receptors was uniform in transmural sections of nonfailing left ventricles (endoocardial:epicardial ratio, 1.0±0.2), whereas in the group of all failing ventricles and in the subgroups with idiopathic or ischemic cardiomyopathy, a significant transmural gradient in the density of β_{1}-receptors was observed (ratios, 0.49±0.07, P<.0001; 0.52±0.11, P<.001; and 0.46±0.09, P<.001, respectively). With the exception of idiopathic dilated ventricles (ratio, 0.76±0.06, P<.05), significant transmural gradients in β_{1}-receptor density were not observed.

Fig 8 shows the ratio of β_{1} : β_{2} receptor density in subepicardial and subendocardial myocytes in control ventricles and in patients with heart failure. In the subepicardium of both control and failing left ventricles, the β_{1}-receptor subtype predominated (β_{1} : β_{2} ratio, 2.33±0.26 and 1.97±0.13, respectively, P=NS). In contrast, β_{1} : β_{2} receptor density ratios were markedly decreased in the subendocardium of failing compared with nonfailing left ventricles (1.04±0.12 versus 2.82±0.45, P<.0001). This decrease was observed in both idiopathic (1.22±0.17, P=.001) and ischemic (0.85±0.14, P<.0001) cardiomyopathy.

**Discussion**

In this study, we used radioligand binding techniques and quantitative light microscopic autoradiography to delineate the distribution of β-adrenergic receptor subtypes in transmural slices of left ventricle in nonfailing control subjects and in patients with severe congestive heart failure. The results demonstrated a 52% reduction in total β-receptor density and a 59% reduction in β_{1}-receptor density in the failing ventricles compared with control hearts. The magnitude of these decreases was similar in patients with idiopathic and ischemic cardiomyopathy. Although similar degrees of β-receptor downregulation have been reported by others, our results differ from those recently reported by Bristow et
Fig 4. Representative autoradiographs showing relative grain densities in subepicardial and subendocardial myocyte regions in nonfailing and failing left ventricles. Transmural sections were incubated with 25 pmol/L ICYP alone (left panels) or in the presence of $10^{-6}$ mol/L L-propranolol (right panels). A, Control subepicardium; B, control subendocardium; C, failing subepicardium; and D, failing subendocardium. Magnification is the same in all panels. Original magnification $\times 400$. Bar=25 $\mu$m.


Fig 5. Bar graphs of specific grain densities in subepicardial (top) and subendocardial (bottom) myocyte regions in control (NF, n=4), failing (F, n=23), idiopathic (IDC, n=12), and ischemic (ISC, n=11) left ventricles. Triplicate transmural sections from each ventricle were incubated with 25 pmol/L ICYP alone; 25 pmol/L ICYP+10^{-7} mol/L CGP-20712A; or 25 pmol/L ICYP+10^{-6} mol/L l-propranolol. At least 10 grain density measurements were made in both regions on each section. Total, β₁, and β₂-specific grain densities were calculated with Equation 1 and corrected as indicated (see text). τP<.05; *P<.001; **P<.0001.

Fig 6. Bar graph of relative specific total β-receptor grain densities in subepicardial and subendocardial myocyte regions of control (NF, n=4), failing (F, n=23), idiopathic (IDC, n=12), and ischemic (ISC, n=11) left ventricles. At least 10 grain density measurements were made in each region in triplicate transmural sections from each ventricle. The ratio of subepicardial to subendocardial grain density measurements was obtained for each section, and the bar graph shows the mean±SEM of the individual ratios. In this way, intersection variability is minimized. *P<.001; †P<.0001 vs a hypothesized mean of 1.0.

Fig 7. Bar graph of relative specific β-receptor subtype grain densities in subepicardial and subendocardial myocyte regions of control (NF, n=4), failing (F, n=23), idiopathic (IDC, n=12), and ischemic (ISC, n=11) left ventricles. At least 10 grain density measurements were made in each region in triplicate transmural sections from each ventricle. The ratio of subepicardial to subendocardial grain density measurements was obtained for each section, and the bar graph shows the mean±SEM of the individual ratios. In this way, intersection variability is minimized. †P<.05; *P<.001; **P<.0001 vs a hypothesized mean of 1.0.
observed no differences in the density of total $\beta$-receptors and $\beta$-receptor subtypes in patients with or without previous $\beta$-agonist therapy. Furthermore, although the nonfailing control hearts were obtained from subjects who had been treated within 24 hours of tissue procurement with intravenous dopamine at concentrations sufficient to exert $\beta_1$-agonist effects, total $\beta$-receptor density was significantly greater in this group compared with either all failing hearts or the subgroups of idiopathic or ischemic cardiomyopathy. Therefore, the altered $\beta$-receptor densities observed in this study do not appear to be attributable to catecholamine therapy.

Treatment with the $\beta_1$-selective antagonist metoprolol has been shown to increase $\beta$-adrenergic receptor density in some patients with idiopathic dilated cardiomyopathy. Such therapy may potentially restore the transmural distribution of receptor subtypes toward normal. However, the mechanism of action underlying the salutary effects of $\beta_1$-blockers in heart failure remains unclear. Although recent results showed benefits in terms of the combined end point of survival and the development of worsening heart failure, there were no obvious survival benefits with this agent.

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