Distribution of β-Adrenergic Receptors in Failing Human Myocardium
Implications for Mechanisms of Down-Regulation

Sidney S. Murphree, MD, and Jeffrey E. Saffitz, MD, PhD

The density of β-adrenergic receptors is reduced in crude membranes prepared from failing human myocardium. We used quantitative autoradiography of radioligand binding sites in intact tissue slices to determine whether the total tissue content of receptors is reduced and to characterize the transmural distribution of receptors in cardiac myocytes and the coronary vasculature in hearts obtained from nine cardiac transplant patients with severe congestive failure. Binding of \(^{125}\)Iodo]cyanopindolol to transmural slices of human myocardium was rapid, saturable, stereoselective, and displaceable by agonists and antagonists with an appropriate rank order of potency. Binding isotherms in four normal and nine failing ventricles showed a significant reduction in the total tissue content of β-receptors in failing myocardium (38.3±2.0 fmol/mg protein) compared with normal tissue (52.4±1.7 fmol/mg protein, p=0.038). In the normal ventricles, the greatest receptor density was observed autoradiographically in myocyte regions of the subendocardium. Receptor density of the coronary arterioles was approximately 70% of that in adjacent myocytic regions. The density of binding sites in both myocytic regions and arterioles was diminished in all regions of the failing ventricles, but down-regulation was due primarily to a selective reduction of β-receptors of subendocardial myocytes (63±5% of subepicardial receptor density vs. 115±6% in controls, p<0.0001). These observations indicate that down-regulation occurs nonuniformly in the transmural distribution and thus is likely not related simply to elevated circulating catecholamine levels. (Circulation 1989;79:1214-1225)

The density of β-adrenergic receptors and responsiveness to β-agonists are reduced in myocardium of patients with congestive heart failure.1–3 The mechanisms responsible for these alterations have not been delineated but may be related to the high levels of circulating catecholamines typically observed in patients with congestive heart failure.

β-Adrenergic receptors appear to reside in both the cell surface membrane (where they may interact with guanine nucleotide–binding proteins to activate adenylate cyclase) and in an intracellular membrane compartment in which coupling of receptor to effector molecules is precluded.4–11 Such diverse factors as agonist-mediated desensitization,7–9 acute ischemia,10 and exposure to antagonists11 may promote redistribution of receptors between the surface and intracellular compartments without necessarily causing a change in the total tissue content of receptors. Redistribution of receptors between surface and internal compartments results in apparent up- or down-regulation of receptor density in the cell surface membrane and the potential for altered responsiveness to adrenergic stimuli.

Previous studies of β-adrenergic receptor density in normal and failing human myocardium have used radioligand binding in conventional membrane preparations (30,000–50,000 g pellet).2,3 These membranes are depleted of the putative internal compartment ("light vesicle fraction") that has a lower buoyant density than membrane fractions enriched in cell surface markers. Thus, the decreased β-adrenergic receptor density in membranes prepared from failing myocardium may reflect sequestration of receptors into the internal compartment without a net loss of receptors rather than reflect a reduction in the total tissue content of receptors. Moreover, use of membranes prepared from homogenates of

From the Department of Pathology and Cardiovascular Division, Washington University School of Medicine, St. Louis, Missouri.

A preliminary report of this work was presented at the Annual Meeting of the American College of Cardiology, March 1987, and was published in abstract form (J Am Coll Cardiol 1987;9:133A).

Supported by National Institutes of Health Grant HL-17646, SCOR in Ischemic Heart Disease. J.E.S. is an Established Investigator of The American Heart Association.

Address for correspondence: Dr. Jeffrey E. Saffitz, Department of Pathology, Box 8118, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110.

Received August 29, 1988; revision accepted February 21, 1989.
whole myocardium precludes analysis of receptor distribution in specific components of the heart such as cardiac myocytes and the coronary vasculature. Recently, we and others have developed and validated methods for autoradiographic localization of β-adrenergic receptors in transmural slices of the heart. With this method, radioligand bound to the total content of receptors in intact tissue slices can be quantified with light microscopic resolution. In the present studies, we have used light microscopic autoradiography to localize the β-adrenergic antagonist [125I]cyanopindolol in transmural slices of myocardium obtained from patients who underwent cardiac transplantation. The studies were designed to address three specific questions. First, is the total tissue content of β-receptors reduced in failing human myocardium? Second, does down-regulation involve only receptors on cardiac myocytes or is the density of vascular receptors reduced as well? Third, does down-regulation occur uniformly in a transmural distribution? Our results indicate that the total tissue content of receptors is reduced in failing myocardium. Although both myocytic and vascular receptor densities are reduced in all regions, down-regulation in the failing heart is related primarily to diminished receptor density in subendocardial myocytes.

Methods

Tissue Procurement

The goal of this study was to compare the transmural distribution of β-adrenergic receptors in myocytic and vascular compartments of normal and failing human myocardium. Normal tissue samples analyzed in the present studies were obtained from the right and left ventricles of a 54-year-old man whose normal heart was harvested for valve homografts and from two cardiac transplant patients in whom one ventricle was functionally and morphologically normal, whereas the other ventricle was severely diseased. In one case, normal tissue was obtained from the left ventricle of a heart-lung transplant recipient with primary pulmonary hypertension in whom preoperative echocardiography showed normal left ventricular wall motion. The cardiac index was 2.8 l/min/m², and the left ventricular end-diastolic pressure was 13 mm Hg. Gross and microscopic examination of the left ventricle revealed no dilatation, hypertrophy, fibrosis, or necrosis. In the second case, normal tissue was obtained also from the right ventricle of a patient with dilated cardiomyopathy whose right ventricular structure and function were normal despite considerable left ventricular disease (right and left ventricular ejection fractions were 67% and 16%, respectively; right and left ventricular end-diastolic pressures were 3 and 23 mm Hg, respectively). Morphologic examination of the right ventricle showed no abnormalities.

Transmural samples of failing myocardium were obtained from hearts of seven additional patients who underwent cardiac transplantation. Table 1 lists selected clinical features of the study patients. Medications at the time of transplantation included angiotensin converting enzyme inhibitors, digoxin, diuretics, and vasodilators. Similar medications had been administered to subjects reported in previous studies. Patients who had been treated with β-agonists or antagonists were excluded from study. Excised hearts were rinsed immediately in saline at 4°C, and transmural blocks of ventricular myocardium were dissected, cooled on dry ice, submerged slowly in liquid nitrogen, and stored in sealed containers at −70°C until used in experiments. Regions of grossly discernable myocardial necrosis or fibrosis were excluded.

Radioligand Binding Assays

Unfixed frozen sections, 12 μm in thickness, were cut with a cryostat-micromtome and mounted on gelatin-coated slides. Two or three serial sections were placed on each slide. Slide-mounted transmural sections of myocardium were incubated at 37°C in buffer (154 mM NaCl, 10 mM MgCl₂, 10 mM Tris-HCl, pH 7.4) containing selected concentrations (0.8–110 PM) of (-)[125I]cyanopindolol (ICYP) (2,200 Ci/mmol; New England Nuclear, Boston, Massachusetts). Nonspecific binding was defined as binding of radioligand in the presence of 10⁻⁵ M l-propranolol. Sections were incubated with radioligand in large volumes of buffer (50–70 ml) so that the concentration of free radioligand did not change measurably during incubation intervals. Non-

---

**TABLE 1. Clinical Features of Cardiac Transplant Recipients**

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>42.0±13.6 (range, 23–60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
</tr>
<tr>
<td>Male</td>
<td>5</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>Idiopathic pulmonary hypertension</td>
<td>1</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>5</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>3</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>2</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
</tr>
<tr>
<td>Duration of symptoms (mo)</td>
<td>17.9±17.0 (range, 4–48)</td>
</tr>
<tr>
<td>Hemodynamic index*</td>
<td></td>
</tr>
<tr>
<td>Cardiac index (l/min/m²)</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>LV ejection fraction (%)</td>
<td>24.5±8.1</td>
</tr>
<tr>
<td>LV end diastolic pressure (mm Hg)</td>
<td>28.3±5.4</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>90.0±6.5</td>
</tr>
</tbody>
</table>

NYHA, New York Heart Association; LV, left ventricle. *Not including hemodynamic data from the patient with idiopathic pulmonary hypertension and normal left ventricular function.
specifically bound radioactivity was removed by incubating tissue sections in buffer without radioligand or unlabeled displacer for selected intervals at 22°C. After rinsing in buffer, sections were dipped briefly in distilled water to remove buffer solutes, dried under a gentle stream of air, and either scraped off slides for quantification of radioactivity with gamma scintillation spectrometry or prepared for autoradiography as described below. Radioactivity in each section was determined by scraping sections from the slides with a razor and quantifying radioactivity in each with gamma scintillation spectrometry (66% counting efficiency). In all experiments, data points were calculated as means of duplicate or triplicate determinations. Radioactivity was normalized to account for variations in section thickness, section size, or amounts of interstitial fibrosis. Total cross-sectional area and tissue protein were measured in groups of sections selected at regular intervals during the preparation of large numbers of serial sections during each individual experiment. Cross-sectional areas were measured by carefully tracing the outlines of enlarged photographs of the sections and digitizing the traced areas. Tissue protein was assessed in individual sections scraped from acid-washed slides (non-gelatin-coated) using the Bio-Rad protein assay and bovine serum albumin standards (Richmond, California). This assay, based on the Bradford dye-binding method, is insensitive to collagen. Thus, effects on apparent receptor density due to variable amounts of interstitial fibrosis in sections of diseased human myocardium were minimized.

**Quantitative Autoradiography**

Radioligand binding sites were localized autoradiographically in transmural myocardial sections with the emulsion-coated coverslip method. Acid-washed, gelatin-coated coverslips were dipped into melted Kodak NTB2 nuclear track emulsion (Eastman Kodak, Rochester, New York) and dried at room temperature for at least 3 hours. The emulsion-coated coverslips were glued at one end to slides containing radiolabeled sections, and after exposure of the emulsion for 18–30 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 4 minutes and fixed with Kodak fixer for 4 minutes at 25°C. After photographic processing, the tissue sections were stained with hematoxylin and eosin, and the coverslips were sealed permanently to the slides. The tissue and overlying developed grains in the emulsion layer were examined by light microscopy. Grain densities were determined by counting grains per unit area of myocytic regions, coronary arterioles (20–70 μm in diameter), and intramural arteries (>300 μm in diameter). Myocytic regions and arterioles of the subepicardium and subendocardium were analyzed separately. For purposes of this analysis, subepicardium and suben-

docardium were defined as regions within five high-power microscope fields of the respective ventricular surface.

Grain densities, determined as number of grains per 1×10⁴ μm² section area, were measured in adjacent serial sections incubated with a saturating concentration (48–50 pM) of ICYP in the presence and absence of 10⁻⁵ M 1-propranolol. Specific grain densities were determined by subtracting grains attributable to nonspecific binding and photographic background, measured in sections incubated with radioligand plus displacer, from those measured in corresponding areas of sections incubated with radioligand only. Because tissue samples were obtained throughout an interval of many months, it was not possible to perform autoradiographic analysis on all samples simultaneously. Variability in autoradiographic conditions (primarily in emulsion exposure intervals) prevented direct comparisons of absolute grain density values except in the two patients in which normal and failing ventricular samples were assessed simultaneously. To facilitate comparisons of β-receptor distribution among all of the normal and failing ventricles analyzed, grain density values of epicardial myocytes were normalized to 1.0, and all other values were expressed as a proportion of this unit value.

Measurements of grain densities of myocytic regions were made only in areas of closely arranged myocytes that were free of significant interstitial fibrosis and artifacts associated with frozen sectioning. Morphometric studies were performed to assess the proportions of myocyte area and sarcolemma length in subendocardial and subepicardial regions in transmural sections of human myocardium. Selected sections were stained with Masson’s trichrome stain to clearly delineate cardiac myocytes (brick red) and interstitial collagen (blue). Regions corresponding to those in adjacent sections used for grain density measurements were analyzed with computer-assisted planimetry. Test regions were displayed on a video monitor, and the perimeter and area of the sectional profile of each cardiac myocyte were measured to determine the aggregate length of cell perimeter (sarcolemma) and area of cardiac myocytes, respectively, in each region. Grain densities were measured in corresponding regions of adjacent sections prepared for autoradiography.

**Statistical Analysis**

Binding isotherm and grain density data were expressed as mean±SEM. Binding isotherm data were transformed by the method of Scatchard. Simple linear regression was used in determining intersections and slopes in Scatchard plots.

The statistical significance of differences in binding isotherm and morphometric measurements was determined with analysis of variance of repeated measures with the SAS general linear models procedures. Differences in grain density measure-
ments in light microscopic autoradiographs were assessed statistically with t tests.

Results

Before undertaking autoradiographic localization of β-adrenergic receptors in human myocardium, preliminary experiments were performed to characterize binding of ICYP to slices of intact tissue. Blocks of tissue obtained from both normal and failing ventricles were sectioned and used to define optimal conditions for removal of nonspecifically bound radioactivity, to determine incubation intervals required to achieve equilibrium binding, and to characterize stereospecificity and the rank order of potency of selected agonists and antagonists in displacing ICYP binding. Because normal human ventricular myocardium was not available in large supply, most of the tissue used in these preliminary experiments was obtained from transplant recipients. No significant differences between normal and failing myocardium were observed in the initial studies described below. Representative experiments shown in Figures 1–3 were performed with failing myocardium.

First, transmural sections were incubated at 37°C with a saturating concentration of radioligand for a prolonged interval (100 pM ICYP for 2 hours) to promote nonspecific binding. Sections were then incubated at 22°C in buffer not containing radioligand or unlabeled displacer for selected intervals, rinsed briefly in distilled water, dried, and scraped for quantification of radioactivity with gamma scintillation spectrometry. As shown in Figure 1, nearly all nonspecifically bound radioactivity was removed during the first 30–60 minutes of rinsing, whereas the amount of specifically bound radioactivity remained constant throughout the 120-minute rinsing interval. Specific binding ratios in excess of 90–95% of total binding were routinely achieved even when saturating radioligand concentrations and prolonged binding intervals were used. Based on these results, 60 minutes of rinsing at room temperature at 20–22°C was used in all subsequent experiments.

The association kinetics of ICYP binding were characterized throughout a broad range of radioligand concentrations to define intervals required to achieve equilibrium binding. As shown in Figure 2, a plateau in specific binding was achieved after incubating sections for approximately 60 minutes in 100 pM (approximately 10×Kd) ICYP at 37°C. At a concentration close to Kd (10 pM), a plateau was achieved after approximately 120 minutes of incubation, whereas at 1 pM, a plateau was difficult to discern although the difference in specific binding at 120 and 180 minutes was not statistically significant. Thus, in subsequent binding isotherm experiments, sections were incubated with radioligand for 90–150 minutes depending upon concentration.

To characterize the stereospecificity of binding and to determine whether binding of ICYP to β-receptors in human left ventricular myocardium could be displaced by agonists and antagonists with a rank order of potency characteristic of β-adrenergic receptors, transmural sections were incubated with ICYP and selected concentrations of the d- and l-stereoisomers of the antagonist propranolol and the agonists isoproterenol and norepinephrine. As shown in Figure 3, the l-stereoisomers were at least two orders of magnitude more potent than the corresponding d-stereoisomers in displacing the...
binding of ICYP. The antagonist l-propranolol was more potent than the agonist l-isoproterenol, which, in turn, was more potent than the agonist l-norepinephrine. The α-adrenergic antagonist phentolamine was ineffective as a displacer of ICYP binding. Thus, binding of ICYP to unfixed transmural sections of human left ventricle was stereoselective and was displaced by antagonists and agonists with a rank order of potency expected of the β-adrenergic receptor.

Representative isotherms and Scatchard plots of radioligand binding to transmural sections of the failing right and the normal left ventricles of the patient with pulmonary hypertension are shown in Figure 4. Composite results of binding isotherm experiments are shown in Figure 5. The total tissue content of receptors (Bmax) in the four normal ventricles was 52.4±1.7 (mean±SEM) fmol/mg protein. Total β-receptor density was reduced significantly in the failing ventricles (Bmax=38.3±2.0 fmol/mg protein, p<0.04). The dissociation constant of radioligand binding (Kd) was 13.9±0.6 pM in normal ventricles and 12.1±0.5 pM in failing ventricles (p<0.02).

The distribution of β-adrenergic receptors in myocytic and vascular components was determined in transmural sections prepared from four normal ventricles and from six failing ventricles in which a sufficient amount of tissue was available for autoradiographic analysis. The results are shown in Figure 6. In the normal ventricles, the ratio of the grain density of subendocardial myocytic regions to that of myocytic regions of the subepicardium was 1.15±0.06 (range, 1.05–1.31). The grain densities overlying small coronary arterioles did not vary significantly in the transmural distribution and were approximately 70% of the density of myocytic regions of the subepicardium. In contrast to the findings in the normal ventricles, the failing ventricles exhibited a striking and consistent diminution in grain density in subendocardial myocytic regions. The failing ventricles showed subendocardial to subepicardial myocytic grain density ratios of only 0.63±0.05 (range, 0.43–0.77). In addition, relative grain densities overlying subendocardial arterioles were significantly lower in failing ventricles than in normal ventricles (0.42±0.07 vs. 0.69±0.07, p<0.04). The grain densities of intramural arteries were considerably lower than those of arterioles and myocytic regions in both normal and failing ventricles. A representative autoradiograph showing the relative grain densities of myocytic regions, arterioles and intramural arteries is shown in Figure 7.

Because all grain density measurements were compared with normalized values of 1.0 assigned to subepicardial myocytes, the data shown in Figure 6 do not indicate the absolute extent of receptor down-regulation in individual compartments but show only that down-regulation occurred in a non-uniform transmural distribution. Table 2 compares normal and failing ventricles in the two patients in which these grain density values were measured simultaneously. In patient 1 with idiopathic pulmonary hypertension, the density of subepicardial myocyte β-receptors was reduced by 30% in the failing right ventricle in comparison with the normal left ventricle. In contrast, the relative reduction in receptor density was twofold greater in subendocardial myocytes. Similar though less striking differences occurred in the regional down-regulation of receptors in subepicardial and subendocardial arterioles (29% and 43%, respectively). In patient 2 (Table 2), β-receptor down-regulation occurred almost exclusively in the subendocardium. Representative autoradiographs of subepicardial and subendocardial myocytic regions of these ventricles are shown in Figures 8 and 9.

Failing human myocardium is characterized morphologically by myocyte hypertrophy and interstitial fibrosis. These structural derangements would tend to decrease myocyte surface-to-volume relations as well as the area density of myocytes. To determine whether regional differences in tissue structure could have contributed to the reduced grain density observed in subendocardial regions of failing ventricles, morphometric analysis was performed in regions corresponding to those in adjacent
FIGURE 4. Plots of binding isotherms (upper panel) and Scatchard plots (lower panel) of $[^{125}]$iodo]cyanopindolol (ICYP) binding to transmural sections of normal left (LV) and failing right (RV) ventricle of a heart-lung recipient with primary pulmonary hypertension. Points are mean±SD of triplicate determinations of specific binding under equilibrium conditions as described in text. $K_d$ and $B_{max}$ values are 13.2 pM and 56.9 fmol/mg protein for the normal LV and 11.2 pM and 24.3 fmol/mg protein for the failing RV.

sections in which grain density measurements were made. Results are shown in Table 3. The proportion of total section area occupied by profiles of cardiac myocytes was equivalent in normal and failing ventricles and did not vary within ventricles in subepicardial and subendocardial regions. Not surprisingly, the amount of myocyte surface membrane (determined by measuring the perimeters of myocyte profiles) per unit section area was diminished in failing ventricles (6.99±0.15 μm/100 μm² compared with 8.41±0.15 μm/100 μm² in normal ventricles, p <0.02). However, this difference was independent of subepicardial or subendocardial location. Thus, differences in β-receptor density observed in subepicardial and subendocardial myocytes within normal and failing ventricles cannot be attributed to regional differences in tissue structure.

Discussion

In this study, we used quantitative light microscopic autoradiography of radioligand binding sites to characterize the distribution of β-adrenergic receptors in myocardium from cardiac transplant recipients with severe congestive heart failure. Results of

FIGURE 5. Plots of binding isotherms in transmural sections of normal (N) and failing (F) myocardium. Individual data points represent the mean of 2–5 determinations of $B_{max}$ and $K_d$ in 1–3 separate transmural tissue blocks from each of four normal and nine failing ventricles. $B_{max}$ is expressed as fmol/mg protein determined in additional sections with the Bradford dye binding method. Group mean±SEM was determined from all individual measurements with analysis of variance of repeated measures.
initial experiments showed that specific binding of ICYP to transmural slices of intact human myocardium was rapid, saturable, of high affinity, and exhibited stereospecificity and the rank order of potency expected of β-adrenergic receptors. Because ICYP has extremely slow dissociation kinetics from β-receptors, rinsing sections for prolonged intervals was possible without removing specifically bound radioactivity. Specific binding ratios greater than 90–95% were routinely achieved, which permitted autoradiographic localization of binding sites with minimal background attributable to nonspecific binding.

The results of binding isotherms indicated that the total tissue content of receptors was diminished...
TABLE 2. Specific Grain Densities of Myocytic Regions and Arterioles in Paired Normal and Failing Ventricles

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal LV Failing</td>
<td>Normal RV Failing</td>
</tr>
<tr>
<td>Subepicardial myocytes</td>
<td>130.8±19.6 (22) 91.1±18.1 (16) 30</td>
<td>152.2±29.9 (61) 135.7±26.2 (26) 11</td>
</tr>
<tr>
<td>Subepicardial arterioles</td>
<td>102.4±29.5 (16) 73.1±14.5 (16) 29</td>
<td>103.2±29.8 (16) 103.8±23.9 (26) 0</td>
</tr>
<tr>
<td>Subendocardial myocytes</td>
<td>172.0±30.0 (23) 70.5±14.3 (14) 59</td>
<td>160.3±42.1 (60) 58.0±20.9 (38) 64</td>
</tr>
<tr>
<td>Subendocardial arterioles</td>
<td>109.9±31.8 (18) 63.0±20.2 (15) 43</td>
<td>101.2±25.3 (17) 73.2±26.7 (22) 28</td>
</tr>
</tbody>
</table>

Values are mean±SD of the number of specific grains/10^4 μm^2 of section area of each compartment. Values in parentheses are the number of individual grain density measurements.

-specific grain densities were determined by subtracting the nonspecific grain density of each compartment (measured in sections incubated with 50 pM [125I]iodo cyanopindolol (ICYP) + 10^−5 M l-propranolol) from total grain density (measured in adjacent serial sections incubated with ICYP only).

in failing myocardium. Previous studies of β-adrenergic receptor content in failing human myocardium have used crude membrane preparations that are typically depleted of the putative internal compartment in which sarcolemmal receptors may be sequestered when surface receptor density is down-regulated. Analysis of the receptor content of crude membranes cannot, therefore, distinguish internalization and net loss of total tissue receptors. Our observations suggest that in severely failing human myocardium such as that obtained from patients undergoing cardiac transplantation, β-adrenergic receptors have been lost, which results in a lower total tissue content. The overall extent of β-receptor down-regulation in failing myocardium observed in the present study (approximately 27%) was less than that reported in previous studies with membranes (approximately 50%). Moreover, part of the decrease in B_max observed in sections of failing myocardium may be attributable to a diminished ratio of membrane surface area to protein in hypertrophied tissue. The disparities between observations in tissue sections and membranes prepared from homogenates suggest that both intracellular sequestration and a net reduction in the total content of receptors may underlie down-regulation. Diminution in the total tissue content of β-adrenergic receptors may be the result of decreased synthesis of receptor protein, increased degradation, or a combination of both mechanisms.

Of the nine failing ventricles studied, eight exhibited diminished receptor density, whereas a single ventricle contained a greater density. This latter sample was obtained from a 37-year-old man who had an anteroseptal infarction with aneurysm formation 4 months before transplantation and had only recently developed severe congestive heart failure. Tissue for receptor analysis was obtained from a markedly hypertrophied area of the posterior left ventricle. Myocardial hypertrophy induced experimentally in laboratory animals is associated initially with increased β-receptor density, but when functionally intact, hypertrophied myocardium becomes dysfunctional and when overt congestive heart failure supervenes, β-adrenergic receptor density and responsiveness generally decline. Thus, the apparent up-regulation of β-receptors observed in one patient may reflect the marked degree of hypertrophy and the recent onset of overt failure in this patient.

In normal ventricles, autoradiography revealed a slightly greater β-receptor density in subendocardial myocytes than in subepicardial myocytes, whereas in failing ventricles, the receptor density of subendocardial myocytes was significantly reduced. Comparison of the distribution of receptors in normal and failing ventricles indicated that while the receptor density of all compartments was diminished in general, the greatest reduction was observed in subendocardial myocytes. Morphometric analysis of the relative amounts of myocyte area and myocyte perimeter (a measure of the amount of sarcolemma) showed conclusively that the autoradiographic observations could not be attributable to regional differences in tissue structure in failing myocardium. Thus, β-adrenergic receptor density is down-regulated nonuniformly in the transmural distribution.

In previous studies of the distribution of β-receptors assessed in membranes prepared from human myocardium, normal tissue was obtained from potential cardiac transplant donors whose hearts were subsequently not used for technical reasons. Unfortunately, our access to fresh, normal donor hearts that were not used in transplantation has been extremely limited. Normal tissues analyzed were obtained from the left and right ventricles of a 54-year-old man whose normal heart was obtained for valve homografts and from two transplant recipients in whom one ventricle was functionally and structurally normal, whereas the other was diseased. We chose not to analyze atrial appendages, papillary muscle fragments from excised valves, or other tissues resected from patients undergoing surgical procedures. In general, such tissues are obtained from abnormal hearts and do not
permit analysis of the transmural distribution of receptors in the ventricles. Despite the limited amount of normal human myocardium analyzed, the results of binding isotherms and autoradiographic studies were highly consistent and fell within a narrow range of values of receptor content and distribution. Moreover, in previous studies of normal canine and feline left ventricle,12,13 a uniform transmural distribution of β-receptors was invariably seen. This observation provides further evi-
dence that the markedly reduced receptor density in subendocardium of failing human myocardium reflects selective down-regulation of $\beta$-receptors in this region.

The mechanisms responsible for selective reduction of receptor density in the subendocardium are not known. Certainly, the observed nonuniform transmural distribution of down-regulation suggests that this process is not related simply to elevated circulating concentrations of catecholamines. A similar conclusion was reached in studies in which $\beta$-receptor densities were compared in membranes prepared from failing right ventricles and normal left ventricles of patients with pulmonary hypertension.2 Although circulating levels of catecholamines are typically increased in patients with congestive heart failure, endogenous myocardial catecholamine stores may be depleted. The effects of alterations in local compared with those of circulating catecholamine levels on myocardial $\beta$-adrenergic receptor density may be disparate.25 Thus, the nonuniform distribution of $\beta$-receptors observed in the present studies in failing myocardium may be related to nonuniform transmural alterations in receptor occupancy by locally released agonist, perhaps in conjunction with altered transmural patterns of sympathetic innervation. Although interactions of receptors with endogenous agonist is likely to be of primary importance in physiologic and pathophysiologic regulation of receptor density and function, other factors relating to differences in blood flow and metabolic demands of the subendocardium in comparison with subepicardium may contribute to down-regulation as well.

Interest has been focused recently on the salutary effects of metoprolol therapy in patients with dilated cardiomyopathy,26,27 and the possibility that such therapy improves cardiac function by up-regulating

Table 3. Morphometric Analysis of Subepicardial and Subendocardial Myocytic Regions

<table>
<thead>
<tr>
<th>Region</th>
<th>% Total area occupied by myocytes</th>
<th>$\mu$m myocyte profile perimeter/100 $\mu$m$^2$ section area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal ventricles</td>
<td>Failing ventricles</td>
</tr>
<tr>
<td>All</td>
<td>89.37±0.67</td>
<td>90.86±0.76</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>89.80±0.93</td>
<td>90.33±1.28</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>88.94±1.03</td>
<td>91.39±0.86</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

In each region, measurements were made in five separate test fields each approximately $2.5 \times 10^4$ $\mu$m$^2$ in area and containing sectional profiles of approximately 40 cardiac myocytes.
myocardial \( \beta \)-receptors. Altered \( \beta_1 \)-receptor density and responsiveness appear to be of particular importance in light of recent results that \( \beta_1 \)-receptors are down-regulated selectively in failing human myocardium resulting in a marked increase in the ratio of \( \beta_1 : \beta \)-receptors. This increased \( \beta_1 : \beta \)-receptor ratio likely accounts for the significantly lower \( K_{D} \) observed in sections of failing tissue with ICYP, which is a modestly \( \beta_2 \)-selective antagonist. The availability of potent, high-specific activity antagonists such as ICYP now makes possible quantitative assessment of \( \beta \)-receptor density in endocardial biopsies either in membranes or autoradiographically in sections. Our results suggest that \( \beta \)-receptor density measurements in endomyocardial biopsies may be sensitive indicators of the extent of up- or down-regulation in the heart because sampling is confined to subendocardial tissue in which altered receptor densities appear to be most marked.

Acknowledgments

We thank James Baker and Timothy Tolley for technical assistance, Kenneth Schechtman, PhD, for statistical assistance, and Susan Johnson for preparation of the typescript. We also acknowledge the generous assistance of R. Morton Bolman, III, MD, Randall E. Genton, MD, Connie Cance, RN, and other members of the Washington University Cardiac Transplantation team. Ayerst Laboratories provided the \( \beta \)-propranolol used in these studies.

References

19. Hoyer D, Engel G, Berthold R: Binding characteristics of (+), (±) and (−)[125I]cyanopindolol to guinea pig left ventricle membranes. Naunyn Schmiedebergs Arch Pharmacol 1982;318:319–329


**KEY WORDS** • β-adrenergic receptor • autoradiography • failing human myocardium • down-regulation
Distribution of beta-adrenergic receptors in failing human myocardium. Implications for mechanisms of down-regulation.
S S Murphree and J E Saffitz

Circulation. 1989;79:1214-1225
doi: 10.1161/01.CIR.79.6.1214

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/79/6/1214