Differences in β-Adrenergic Neuroeffector Mechanisms in Ischemic Versus Idiopathic Dilated Cardiomyopathy

Michael R. Bristow, MD, PhD; Frederick L. Anderson, MD; J. David Port, PhD; Lisa Skerl, BS; Ray E. Hershberger, MD; Patti Larrabee, BS; John B. O'Connell, MD; Dale G. Renlund, MD; Kirk Volkman, RN; June Murray, RN; and Arthur M. Feldman, MD, PhD

Background. We measured the content and activities of components of the β-adrenergic receptor–G protein–adenylate cyclase complex and adrenergic neurotransmitter levels in left and right ventricular myocardial preparations derived from 77 end-stage failing human hearts from patients with idiopathic dilated cardiomyopathy (IDC) or ischemic dilated cardiomyopathy (ISCDC).

Methods and Results. The results were compared with data obtained in 21 nonfailing hearts removed from organ donors. Compared with ISCDC ventricles, IDC left and right ventricles exhibited a greater degree of total β- or β1-receptor down regulation. In contrast, compared with IDC right ventricles, isolated tissue preparations of ISCDC right ventricles exhibited a greater degree of subsensitivity to the inotropic effect of isoproterenol, indicating a relatively greater degree of functional uncoupling of right ventricular ISCDC β-receptors from mechanical response. In addition, relative to IDC left ventricles, preparations of ISCDC left ventricles exhibited greater subsensitivity to β-agonist-mediated adenylate cyclase stimulation, indicating functional uncoupling of left ventricular ISCDC β-receptors from cyclic AMP generation. The uncoupling of β-receptors in ISCDC left and right ventricles may have been a result of abnormalities in G protein activation of adenylate cyclase; compared with age- and cardiac function-matched respective left or right IDC ventricles, ISCDC left ventricles exhibited less stimulation of adenylate cyclase by NaF or forskolin but no change in Mn2+ stimulation, whereas ISCDC right ventricles exhibited less stimulation by the nonhydrolyzable guanine nucleotide Gpp(NH)p. Also, IDC right ventricles exhibited a “selective” (not present in IDC left ventricles or ISCDC ventricles) decrease in stimulation of adenylate cyclase by Mn2+. Tissue neurotransmitter levels and pertussis toxin–catalyzed ADP ribosylation were altered to similar extents in IDC and ISCDC.

Conclusions. These data indicate that potentially important differences exist in the regulatory behavior of components of the β-adrenergic receptor–G protein–adenylate cyclase complex in IDC versus ISCDC, differences that presumably relate to the distinct pathophysiologies of these two types of heart muscle disease. (Circulation 1991;84:1024–1039)
heart has been from subjects with IDC. Because only limited data have appeared on other types of heart muscle disease, we elected to examine the most prevalent kind of cardiomyopathy in most industrialized societies, "ischemic" or postinfarction cardiomyopathy (ISCDC) resulting from coronary artery disease. The data indicate that potentially important differences exist in heart failure-associated changes in the β-adrenergic receptor–G protein–adenylate cyclase complex in ISCDC versus IDC.

Methods

Tissue Procurement

Nonfailing human ventricular myocardium (group A) was obtained from the left and right ventricles of 21 brain-dead organ donors, (age, 5–58 years) for whom no suitable cardiac recipients were available. These donors had been maintained on respirators for 1–3 days, and they had not been given β-adrenergic agonists other than dopamine. In nine of the 21 organ donors, dopamine was administered for peripheral vascular and renal function support at dosages of 3–40 μg/kg/min (median, 10.0 μg/kg/min) for 1–38 hours (median, 9 hours). The nine organ donors who received dopamine did not differ from the 12 who had not received dopamine in any of the measured parameters except for tissue dopamine level, which was higher in those who received dopamine (903 ± 111 versus 318 ± 73 ng/g tissue in those who had not received dopamine, p<0.01 for combined left and right ventricular chambers), and tissue neuropeptide Y level, which was lower in patients who had received dopamine (76.7 ± 5.5 versus 111 ± 11.4 pg/mg protein in those who had not received dopamine, p<0.01). Thus, all organ donor controls were used in the analysis without regard for dopamine administration. No organ donor control had a history of heart disease, and all had normal global left ventricular function by echocardiography performed as part of the organ donation screening process. In all cases, written consent for organ donation for research purposes was obtained from a family member, and all donors were procured locally.

Failing human ventricular myocardial tissue was obtained at the time of cardiac transplantation from the mid free wall of left and right ventricles and right ventricular trabeculae of 49 patients with end-stage biventricular failure resulting from IDC (group B) (age range, 10–61 years). In addition, failing human ventricular myocardial tissue was obtained at the time of cardiac transplantation from the noninfarcted (no visible fibrosis) areas of the mid free walls of left and right ventricles and right ventricular trabeculae of 28 patients with end-stage biventricular failure resulting from ISCDC (group C) (age range, 32–63 years). Of these 28 hearts with ISCDC, 67.9% had three-vessel disease defined as more than 50% arteriographic narrowing of a major (supplying more than 10% of left ventricular myocardium) epicardial coronary artery, 28.6% had two-vessel disease, and one patient (3.6%) had one-vessel disease. Of the three primary coronary arteries, 92.9% of patients had involvement of the left anterior descending coronary artery, 85.7% had involvement of the left circumflex artery, and 85.7% had involvement of the right coronary artery. Heart failure symptoms (e.g., fatigability, breathlessness) were the predominant complaints leading to transplantation in all 28 group C subjects. No group B or C patient had received a β-agonist, a β-antagonist, or a phosphodiesterase inhibitor within 4 weeks of undergoing transplantation, and none had ever received amiodarone. All group B and C subjects were receiving digoxin and diuretics, and 84% were receiving angiotensin converting enzyme inhibitors.

Both nonfailing and failing hearts were rapidly removed, placed in ice-cold oxygenated Tyrode's solution, and transported immediately to the laboratory as previously described. Briefly, 2-g aliquots of left and right ventricular myocardium were removed from the explanted heart of cardiac transplant recipients or prospective donors, placed in ice-cold oxygenated Tyrode's solution, weighed, and homogenized in an ice-cold solution of 250 mM sucrose, 5 mM Tris, and 1 mM EGTA buffer (pH 7.5). The washed particulate fraction was frozen in this same buffer and stored at −70°C for periods of 2 days to 4 months until use. This gently handled particulate fraction exhibits approximately twice as much β-agonist stimulation as the mechanically disrupted crude membrane preparation used for receptor binding.

The "standard" assay condition consisted of 75–250 μg membrane protein in 100 mM Tris buffer (pH 7.30 at 30°C) containing 0.1 mM Mg-ATP, 0.5 mM MgCl2, 10 μM GTP, 1 mM cyclic AMP (cAMP), [3H]cAMP, 10 mM phosphocreatine, and 1.75 units creatine kinase. A second assay condition, termed the Gpp(NH) p condition, consisted of the above mixture minus GTP plus 10−6 M (−)-propranolol. A third assay condition was used to assess Mn2+ stimulation (Mn2+ condition) and consisted of the Gpp(NH) p condition minus MgCl2. After a 5-minute warm-up period, measurement of adenylate cyclase activity was begun by adding 1.0–2.5 μCi [α-32P]ATP (New England Nuclear, Boston). After incubation at 30°C for 20 minutes (the time to reach steady state for hormone stimulation), the reaction was stopped by the addition of 1% sodium dodecyl sulfate (SDS). Formed [32P]cAMP was then isolated by dual-column chromatography. The methods yielded reagent blanks that were less than 10% of basal activity in all cases, and recovery of [3H]cAMP was 70–95%. Results were expressed as net stimulation (ligand stimulation minus basal activity defined as activity with H2O used instead of an agonist) or the "stimulation ratio" of net ligand stimulation to net stimulation by 10 mM NaF.
Creatine Kinase

Creatine kinase activity was measured by a spectrophotometric technique as previously described, with activity expressed in international units per gram of wet weight.

β-Receptor Radiolabeling

Total β-adrenergic receptor density was assessed by $^{125}$I[iodocyanopindolol (ICYP) binding, as previously described. Crude membranes were prepared from a 5-g aliquot of left ventricular free wall by contractile protein extraction and washing a 50,000g pellet, as previously described. The contractile protein–extracted, crude membrane preparation contains more than 90% of the receptors present in the initial homogenate and has twofold to threefold enrichment in β-receptors and other membrane markers. The binding parameters $B_{max}$ and $K_D$, were determined by nonlinear least-squares methodology as previously described. In addition, the proportion of $\beta_1$ versus $\beta_2$-receptors was assessed by betaexol-ICYP or CGP 20712A–ICYP competition curves, with the proportion of $\beta_1$ and $\beta_2$-receptors and their $K_D$ values determined by computer modeling. Both $\beta_1$ and $\beta_2$-receptor densities were determined by multiplying their respective fractions by the total β-receptor density measured by ICYP saturation curves. In competition curve measurements, the concentration of ICYP was 50 pM for ICYP $K_D$ values of less than 20 pM and 100 pM if the ICYP $K_D$ from saturation curves was more than 20 pM; receptor concentration was 2–5 pM. Additional equilibrium assay conditions for ICYP binding were as described previously.

Toxin-Catalyzed ADP Ribosylation

G protein substrates of pertussis toxin were assayed using pertussis toxin–catalyzed incorporation of $^{32}$P ADP-ribose as previously described. In brief, 50 µg of the particulate fraction described for the adenylate cyclase assay was incubated for 90 minutes at 30°C in 100 µl of 100 mM Tris-Cl (pH 8.0) containing 6 mM MgCl$_2$, 2 mM GTP, 10 mM thymidine, 2.5 mM ATP, 10 mM isoniazid, 10 µM $^{32}$P NAD (10 Ci/mmole), and activated pertussis toxin. Pertussis toxin was activated by dialyzing with 10 mM Tris Cl (pH 8.0) for 1 hour at 4°C and then incubating in 100 mM dithiothreitol (DTT) for 30 minutes at room temperature. The reaction was stopped by centrifugation at 15,000g for 5 minutes and washing the pellet with ice-cold 50 mM Tris-Cl (pH 8.0) containing 5% (wt/vol) sucrose, 6 mM MgCl$_2$, 1 mM EDTA, and 1 mM DTT.

G protein substrates of cholera toxin were assayed in an analogous fashion. Membranes (100 µg) were incubated for 90 minutes at 30°C in 100 mM KPO$_2$ (pH 7.0) buffer containing 2 mM GTP, 2.5 mM ATP, 20 mM thymidine, 20 mM arginine, 10 IU aprotinin, 10 µM $^{32}$P NAD (20 Ci/mmole), and 5 µg activated cholera toxin. Cholera toxin was activated by incubating in 10 mM DTT for 30 minutes at 30°C. The reaction was stopped like in the pertussis toxin–catalyzed reactions, and the membrane pellets were resuspended in 50 µl of a solution containing 62.5 mM Tris-Cl (pH 6.8), 2% SDS, 10% glycerol, and 5% β-mercaptoethanol. Proteins were electrophoretically separated on a 7.5% SDS–polyacrylamide gel. Gels were dried on cellophane and imaged by exposure to Kodak XAR-5 film with an intensifying screen at −70°C for 24–72 hours.

The signal intensity of the appropriate bands on the autoradiograms was analyzed using a computer-assisted two-dimensional densitometer (Loates, Westminster, Md.). Autoradiographic measurements were corrected for total protein in each lane by using the Coomassie blue–stained proteins on the same gels. Four randomly selected Coomassie blue–stained protein bands were quantified using two-dimensional transmission densitometry as previously described. Autoradiographic densities obtained from separate experiments were standardized by including membranes from at least three nonfailing hearts in each experiment. The levels of the ADP-ribosylated proteins in membranes from failing hearts were then calculated as a percentage of the mean of the nonfailing controls. This method of comparing G protein levels in the same tissue from different individuals has been used extensively by us and others. Because each sample was assayed multiple times in separate experiments and the values for each of these measurements were averaged, the mean of the controls does not equal 100%.

Tissue Catecholamine and Neuropeptide Y Levels

Tissue catecholamine and neuropeptide Y levels were measured in 100–200 mg quick-frozen (liquid nitrogen) tissue aliquots from freshly excised left and right ventricular free wall and stored at −70°C until used. For catecholamine determinations, tissue was extracted with 0.4 M perchloric acid containing 5 mM glutathione, and the soluble portion was analyzed by a radioenzymatic technique (Amersham, Arlington Heights, Ill.). Tissue extracts were diluted 1:10 and 1:50 before assay, and the reported values for noradrenaline, epinephrine, and dopamine are the averages of the determinations in each of these dilutions.

Neuropeptide Y levels were measured with a radioimmunoassay by using a rabbit polyclonal antibody that did not cross react (less than 1% activity) with multiple peptides having significant homology with neuropeptide Y. A homogenate of 40–60 mg of tissue (left or right ventricular free wall) that had been stored at −70°C was initially minced and boiled in 0.01N HCl, with half of the material being used for protein measurements and the other half for the neuropeptide Y assay. Results are expressed in picograms of neuropeptide Y per milligram of protein.
TABLE 1. Clinical Data

<table>
<thead>
<tr>
<th>Patient group (n)</th>
<th>Age (yr)</th>
<th>Male (%)</th>
<th>EF (%)</th>
<th>CI (l/min/m²)</th>
<th>Pressure (mm Hg)</th>
<th>New York Heart Association functional class</th>
<th>Duration of heart failure symptoms (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (nonfailing) (n=21)</td>
<td>33.6±3.5 (5–58)</td>
<td>43</td>
<td>. . .</td>
<td>. . .</td>
<td>. . .</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>B (failing, idiopathic dilated cardio-myopathy) (n=49)</td>
<td>38.9±2.2* (10–62)</td>
<td>82</td>
<td>15.6±0.9 (8–33)</td>
<td>2.08±0.11 (0.8–3.5)</td>
<td>21.8±1.1 (10–35)</td>
<td>30.0±1.6 (11–50)</td>
<td>8.1±1.1 (0–35)</td>
</tr>
<tr>
<td>C (failing, ischemic dilated cardio-myopathy) (n=28)</td>
<td>52.0±1.6* (32–63)</td>
<td>96*</td>
<td>16.3±0.9 (10–27)</td>
<td>2.20±0.11 (1.1–3.9)</td>
<td>20.6±1.7 (4–39)</td>
<td>26.6±2.4 (12–53)</td>
<td>5.6±1.0 (1–20)</td>
</tr>
</tbody>
</table>

EF, ejection fraction; CI, cardiac index; PW, mean pulmonary wedge pressure; PAP, mean pulmonary artery pressure; RA, mean right atrial pressure.

*p<0.05 vs. A.

Values are given as mean±SEM (range).

Isolated Muscle Response

Mechanical responses of isolated right ventricular trabeculae of uniform size (1–2×6–8 mm) harvested from freshly explanted organ donors or transplant recipients were measured as previously described.1,10,14 Briefly, trabeculae were mounted in an eight-chamber muscle bath containing Tyrode’s solution1,8 bubbled with 95% O₂–5% CO₂ to give final pH 7.45. Trabeculae were field-stimulated at 10% above threshold, and resting tension was set at the length that produced the maximum degree of systolic contraction, usually approximately 1 g. After a 2-hour equilibration period, full dose–response curves to isoproterenol were performed using 0.5 log unit dose increments between 10⁻⁹ and 10⁻³ M. After completion of the isoproterenol dose–response curve and washout of isoproterenol, the maximal response to calcium was measured by administering calcium chloride at final concentrations of 5 and 10 mM. Tension was recorded as the stimulated tension minus baseline tension, and the maximum response was taken as the highest net tension produced at any point in the dose–response curve.

Statistical Analysis

The method of analyzing radioligand-unlabeled ligand competition curves has been previously described,1 as has the analysis of radioligand saturation binding curves.1,3 Differences between more than two groups were assessed by analysis of variance (ANOVA) and the Scheffe F test. Differences between two groups were assessed by Student’s t test, with a probability value of less than 0.05 in a two-tailed distribution considered statistically significant. Polynomial and simple regression analyses between β-adrenergic receptors and tissue catecholamines were performed on a Macintosh IIci computer using the STATVIEW 512+ program (Brain Power, Inc., Calabasas, Calif.), which was also used for the ANOVA and t tests.

Source of Compounds and Reagents

ICYP was purchased from Amersham. [³²P]ATP, [³²P]ADP-ribose, and [¹²⁵I]neuropeptide Y were purchased from New England Nuclear, Boston. Betaxolol was a gift from Synthelabs (L.E.R.S.), Paris; CGP 20712A was provided by CIBA-GEIGY, Summit, N.J.; and (−)-propranolol was a gift from Ayerst Laboratories (New York, N.Y.). Zinterol was obtained from Bristol Myers, Evansville, Ind. All other chemicals were purchased from standard commercial suppliers.

Results

Clinical Data

Experimental material was divided into three groups: group A, nonfailing controls; group B, IDC; and group C, ISCDC. As can be seen in Table 1, patients with ISCDC were older than subjects with IDC or nonfailing controls. In addition, nonfailing controls had a lower incidence of male gender – 43% versus 82% in IDC and 96% in ISCDC. However, analyses of receptor, neurotransmitter, and muscle contraction data with regard to gender revealed no differences between groups A and B.

As shown in Table 1, catheterization and ejection fraction data for group B versus group C revealed no significant differences in global ventricular function. However, all hemodynamic parameters tended to be more abnormal in subjects with IDC. New York Heart Association functional class and duration of heart failure symptoms did not differ between groups B and C.

β-Adrenergic Receptor Densities and Subtype Fractions

Left ventricles. Shown in Table 2 are β-receptor data for the three groups. Relative to nonfailing controls (group A), total β-receptor density was reduced in both the IDC (group B) and ISCDC (group C) left ventricles. The reduction in left ventricular total β-receptor density was greater in group B than group C, because group C was significantly different from either group A or B. Left ventricular ICYP Kᵦ values did not differ among the three groups. The decrease in total β-receptor density in each group resulted from a decrease in left ventric-
for the document.
**Table 3.** Tissue Catecholamine, Neuropeptide Y, and Creatine Kinase Levels

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Group A (nonfailing controls)</th>
<th>Group B (idiopathic dilated cardiomyopathy)</th>
<th>Group C (ischemic dilated cardiomyopathy)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LV (n=13)</td>
<td>RV (n=13)</td>
<td>LV + RV (n=26)</td>
</tr>
<tr>
<td><strong>Catecholamine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>10.3±1.2*</td>
<td>10.1±1.0*</td>
<td>20.4±1.3*</td>
</tr>
<tr>
<td>Dopamine</td>
<td>11.2±1.5*</td>
<td>11.0±1.2*</td>
<td>22.4±1.4*</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>12.1±1.0*</td>
<td>11.9±1.0*</td>
<td>23.2±1.2*</td>
</tr>
<tr>
<td>Neuropeptide Y</td>
<td>80±175</td>
<td>78±168</td>
<td>158±183</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>10.3±0.8</td>
<td>10.1±0.7</td>
<td>20.4±1.3*</td>
</tr>
</tbody>
</table>

LV, left ventricles; RV, right ventricles.

Adenylate Cyclase Stimulation

**Left ventricles.** Table 4 gives adenylate cyclase data for the three groups. Under standard assay conditions, stimulation by the β-agonists isoproterenol and zintrol was reduced only in group C. Forskolin stimulation was also reduced in group C. The ratios of isoproterenol or zintrol stimulation to NaF were reduced in both groups B and C and to similar extents.

The response to Gpp(NH)p was reduced in both groups B and C. The Gpp(NH)p-to-NaF ratio was also reduced in both cardiomyopathy groups. In contrast, the response to Mn²⁺ was not reduced in either group B or C.

**Right ventricles.** Under standard assay conditions, basal activity was significantly reduced only in group B. The responses to isoproterenol, zintrol, NaF, and forskolin were not significantly reduced in either

**Table 4.** Adenylate Cyclase Data (pmol cyclic AMP/min/mg as Net Stimulation [Ligand Stimulation Minus Basal Activity] or Stimulation Ratio)

<table>
<thead>
<tr>
<th>Standard condition (n)</th>
<th>Group A (nonfailing controls)</th>
<th>Group B (idiopathic dilated cardiomyopathy)</th>
<th>Group C (ischemic dilated cardiomyopathy)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LV (n=20)</td>
<td>RV (n=20)</td>
<td>LV + RV (n=40)</td>
</tr>
<tr>
<td>Basal activity</td>
<td>100±7.4</td>
<td>100±7.4</td>
<td>100±7.4</td>
</tr>
<tr>
<td>Isoproterenol maximum</td>
<td>30±3.0</td>
<td>30±3.0</td>
<td>30±3.0</td>
</tr>
<tr>
<td>NaF (10⁻² M)</td>
<td>45±4.5</td>
<td>45±4.5</td>
<td>45±4.5</td>
</tr>
<tr>
<td>Forskolin (10⁻³ M)</td>
<td>22±2.2</td>
<td>22±2.2</td>
<td>22±2.2</td>
</tr>
<tr>
<td>Isoproterenol/NaF</td>
<td>0.8±0.05</td>
<td>0.8±0.05</td>
<td>0.8±0.05</td>
</tr>
<tr>
<td>Zintrol/NaF</td>
<td>0.2±0.02</td>
<td>0.2±0.02</td>
<td>0.2±0.02</td>
</tr>
</tbody>
</table>

LV, left ventricles; RV, right ventricles.

Values are given as mean±SEM.

Isoproterenol and zintrol maximum stimulations are from full dose–response curves (10⁻⁸ to 10⁻⁴ M each).

*Paired t, p<0.05 vs. LV.
group B or C. However, the isoproterenol and zinterol responses normalized to NaF stimulation were significantly reduced in both groups B and C.

The response to Gpp(NH)p and the Gpp(NH)p-to-NaF ratio were significantly reduced only in group C. In contrast, the response to Mn2+ was reduced in group B but not in group C.

**Left ventricles plus right ventricles.** When left and right ventricles were pooled, the standard assay condition data yielded significant reductions in basal activity in groups B and C, zinterol stimulation in both groups B and C, forskolin stimulation in groups B and C, and isoproterenol and zinterol responses normalized to NaF in groups B and C.

The response to Gpp(NH)p and the Gpp(NH)p-to-NaF ratio were reduced in both groups B and C. The Mn2+ response tended to be reduced in group B, but the result did not achieve statistical significance versus group A or C.

**Left versus right ventricles.** In group A, the NaF response was significantly lower in right than in left ventricles, which contributed to significantly higher isoproterenol-to-NaF and zinterol-to-NaF ratios in this group. Under the Gpp(NH)p condition, the response to Gpp(NH)p and the Gpp(NH)p-to-NaF ratio were significantly lower in right ventricles. The response to NaF was also reduced in group A right ventricles compared with left ventricles. Stimulation by Mn2+ in left ventricles was not different from that in right ventricles.

In group B, under standard assay conditions, basal activity and the response to isoproterenol were lower in right ventricular preparations. There were no other differences in left versus right ventricular activities of adenylate cyclase in group B.

In group C, under standard assay conditions, the responses to isoproterenol, zinterol and forskolin and the zinterol-to-NaF ratio were significantly lower in left than in right ventricles. There were no differences in Gpp(NH)p or Mn2+ stimulation in group C left and right ventricles.

**Toxin-catalyzed ADP ribosylation.** Table 5 shows relative values for cholera toxin- and pertussis toxin-catalyzed ADP ribosylation expressed as a percentage of nonfailing controls. As can be seen, particulate fractions prepared from the left ventricles of patients with IDC or ISCDC exhibited increased activities of pertussis toxin-catalyzed ADP ribosylation, with no difference between groups B and C. Cholera toxin-catalyzed ADP ribosylation was not different among the three groups.

**Tissue bath responses to isoproterenol and calcium.** Shown in Table 6 are responses of isolated right ventricular trabeculae to isoproterenol and calcium. Relative to group A, isoproterenol responses were decreased in groups B and C. The response to isoproterenol normalized to each individual strip's calcium response (isoproterenol-to-calcium ratio) was also decreased in both groups B and C, with group C tending to have a greater reduction (reduced by 50% in C versus 37% in B). Responses to calcium did not differ among the three groups.

**Age-Restricted Analysis**

Because group C patients were significantly older than those in group A or B (see Table 1), an age-adjusted analysis of the three groups was performed by restricting the analysis of groups A and B to patients 40 years old or older (Table 7), the 50th percentile of ages in group B. As can be seen in Table 7, this restriction yielded near-identical ages among the three groups. Like in the unrestricted analysis, left and right ventricular tissue behaved similarly for all parameters measured. The differences in total β- and β1-receptor densities between groups B and C noted in Table 2 remain, as does the lack of differences in neurotransmitter levels. Because of a lower total β-receptor density in group A, β-receptor density in group C was no longer significantly reduced in any chamber, whereas group C β1-receptor density was significantly reduced (by 24%) only in the combined (left plus right ventricles) analysis. The tissue bath response to isoproterenol was significantly reduced only in group C, although the isoproterenol-to-calcium ratio was reduced in both groups.

Adenylate cyclase stimulation in the age-restricted analysis generally revealed the same trends and differences noted in Table 4. As shown in Table 6.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Isoproterenol</th>
<th>Calcium</th>
<th>Isoproterenol-to calcium ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (nonfailing, organ donors; n=36 trabeculae)</td>
<td>1,912±223</td>
<td>1,200±179</td>
<td>2.02±0.25</td>
</tr>
<tr>
<td>B (failing, idiopathic dilated cardiomyopathy; n=77 trabeculae)</td>
<td>1,366±106*</td>
<td>1,150±90</td>
<td>1.28±0.10*</td>
</tr>
<tr>
<td>C (failing, ischemic dilated cardiomyopathy; n=55 trabeculae)</td>
<td>1,002±83*</td>
<td>1,171±94</td>
<td>1.02±0.08*</td>
</tr>
</tbody>
</table>

*Groups B and C: p<0.05 (F) and significant Scheffe test vs. group A.

Values are given as maximum stimulation minus baseline tension±SEM.
in Table 7, relative to adenylate cyclase stimulation by NaF, group B and C ventricles exhibited similar decreases in responsiveness to isoproterenol, zinterol, and Gpp(NH)p that were statistically significant in the left-plus-right-ventricles analysis. Like in the unrestricted analyses, the Gpp(NH)p response tended to be lower in group C (reduced by 33%) right ventricles than in group B right ventricles (reduced by 16%), but neither reduction achieved statistical significance. In addition, individual left or right ventricular Gpp(NH)p-to-NaF ratios were significantly reduced only in group C. Because of the smaller sample size, isoproterenol stimulation of adenylate cyclase was no longer reduced in group C left ventricles, but the respective values relative to group B left ventricles exhibited the same quantitative patterns (isoproterenol value, 95% of nonfailing control in group B and 78% of control in group C) as in the unrestricted data. Forskolin and NaF stimulation also tended to be lower in ISCDC left ventricles than in IDC left ventricles, but the respective values were not reduced below those of nonfailing controls.

Similarly, Mn2+ stimulation of adenylate cyclase was no longer significantly reduced in group B right ventricles (data not shown), but the percent reduction (by 35%) was identical to that in the unrestricted analysis. The only hemodynamic variable that tended to differ between groups B and C after imposition of the age restriction was mean pulmonary artery pressure (31.6±2.0 mm Hg in group B versus 26.8±2.3 mm Hg in group C, p=0.13).

**Age- and Cardiac Function–Restricted Analyses**

Because mean pulmonary artery pressure was marginally different in the age-restricted group, an additional analysis was made by excluding age-restricted patients in group B with mean pulmonary artery pressures of more than 35 mm Hg, the 62.5 percentile in this group. This restriction yielded a subset of group B and C patients with essentially identical ages and hemodynamics, with values in IDC for age of 50.1±1.2 years; ejection fraction, 16.3±1.1%; cardiac index, 2.45±0.19 l/min/m²; mean pulmonary wedge pressure, 19.2±1.7 mm Hg; mean right atrial pressure, 5.9±1.2 mm Hg; and mean pulmonary artery pressure, 26.1±1.4 mm Hg (all p=NS versus ISCDC data listed in Table 1).

As can be seen in Figure 1, the differences in total β- and β2-receptor densities between groups B and C noted in Table 2 persist after adjustment for age and hemodynamics. As shown in Figure 1, in pooled left ventricles plus right ventricles, total β- and β2-receptor densities were 24% and 33%, respectively (both p<0.05) higher in group C than in group B, whereas tissue neurotransmitters, creatine kinase levels, and the NaF-normalized stimulation of adenylate cyclase by β-agonists were not different in the two groups. Although the Gpp(NH)p-to-NaF ratio tended to be lower in ISCDC ventricles, the difference did not achieve statistical significance (p=0.090).

Figure 2 gives the basal activity and various forms of adenylate cyclase stimulation in left or right ventricles for the age- and pulmonary artery pressure–restricted analysis. In left ventricles, it can be seen that stimulation of adenylate cyclase by isoproterenol tended to be more blunted in the ISCDC group than in the IDC group (p=0.062), with the NaF and forskolin responses significantly reduced in ISCDC compared with IDC. Stimulation by Gpp(NH)p or Mn2+ in the two groups was not different. In contrast, the only type of stimulation in right ventricles that was different in group B compared with group C was the Gpp(NH)p response, which was significantly lower in group C. In addition, the Gpp(NH)p ratio almost achieved statistical significance (0.28±0.04 in group B and 0.20±0.02 in group C, p=0.071) in right ventricles.

Because the contractile response was assessed only in right ventricular preparations, Figure 3 was constructed to compare the behavior of right ventricular

**FIGURE 1.** Bar graph of changes in β-adrenergic receptor–G protein–adenylate cyclase complex parameters and neurotransmitters in ischemic dilated cardiomyopathy (IDC) versus idiopathic dilated cardiomyopathy (ISCDC) in pooled left plus right ventricles (LV+RV). IDC material is age restricted to 40 years or more and pulmonary artery pressure (PAP) restricted to 35 mm Hg or less. Data are expressed as percent of values in age-restricted nonfailing controls (see Table 7). B TOT, total β-receptor density; B-1, β1-receptor density; B-2, β2-receptor density; NE, tissue norepinephrine; NPY, neuropeptide Y; CK, creatine kinase; I/F CYC, ratio of maximum net adenylate cyclase stimulation by isoproterenol to stimulation by 10−2 M NaF; Z/F CYC, ratio of maximal net adenylate cyclase stimulation by zinterol to stimulation by 10−2 M NaF; G/F CYC, ratio of maximal net adenylate cyclase stimulation by 10−4 M Gpp(NH)p to stimulation by 10−2 M NaF.
TABLE 7. Comparison of Various Parameters With Groups A and B Restricted to Subjects 40 Years Old or Older

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Age (years)</th>
<th>Ventricles</th>
<th>Receptor density (fmol/mg)</th>
<th>Neurotransmitter tissue level (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (nonfailing; n=7 subjects, 14 chambers)</td>
<td>52.1±1.6</td>
<td>LV</td>
<td>Total $\beta$, $\beta_1$, $\beta_2$</td>
<td>NE (ng/g), NPY (pg/mg), CK (IU/g)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RV</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LV</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LV + RV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (idiopathic dilated cardiomyopathy; n=26 subjects, 52 chambers)</td>
<td>50.1±1.2</td>
<td>RV</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LV</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LV + RV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (ischemic dilated cardiomyopathy; n=28 subjects, 55 chambers)</td>
<td>51.3±1.5</td>
<td>RV</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LV</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LV + RV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NE, norepinephrine; NPY, neuropeptide Y; CK, creatine kinase; LV, left ventricles; RV, right ventricles.

$n$ values in contractile response data refer to number of individual trabeculae.

*p < 0.05 vs. group A.

†p < 0.05 vs. group B.

Values are given as mean±SEM.

receptors with physiological response. As can be seen, the differences in total $\beta$- and $\beta_1$-receptor densities noted in Figure 1 are also present in right ventricular tissue. Total $\beta$-receptor density in ISCDC right ventricles was 28% higher than in IDC right ventricles ($p<0.05$) compared with 24% higher in ISCDC for the left-plus-right-ventricles group ($p<0.05$) and 20% higher in ISCDC for the left ventricular group ($p<0.10$). Similarly, $\beta_1$-receptor density in ISCDC right ventricles was 33% higher ($p<0.10$) than in IDC right ventricles compared with an identical (33%) higher value for left ventricles or combined left plus right ventricles (both $p<0.05$) in the ISCDC group. Despite the higher total $\beta$-receptor density in group C compared with group B, the isoproterenol-mediated contractile response was significantly more blunted in group C (by 37%, $p<0.001$). The calcium response did not differ in the two groups, whereas the isoproterenol-to-calcium ratio was 20% lower in group C ($p<0.05$).

Relation Between $\beta$-Receptor Densities and Tissue Catecholamines in Groups B and C

Tissue norepinephrine depletion, as an index of adrenergic activity, should correlate with $\beta_1$-receptor downregulation if the latter is a result of increased cardiac adrenergic drive. Figure 4 gives the relation between $\beta_1$- or $\beta_2$-adrenergic receptors and tissue norepinephrine levels in both groups of dilated cardiomyopathy.

Figure 2. Bar graph of basal (BSL) activity and net stimulation (maximal stimulation minus basal activity) of adenylate cyclase (A.C.) in left ventricles (LVs) or right ventricles (RVs) of group B and C ventricles in age- and pulmonary artery pressure (PAP)-restricted analyses. First (left to right) five sets of bar graphs were assayed under standard conditions, Gpp(NH)p (GGPNHP) was assayed under "Gpp(NH)p" conditions, and Mn? (Mn) was assayed under "Mn?" conditions. Forskolin (FORSK) stimulation is 0.1 of actual value to allow data to be presented on the same scale. ISO, isoproterenol; NPY, neuropeptide Y; ZINT, zinterol; cAMP, cyclic AMP; IDC, idiopathic dilated cardiomyopathy; ISCDC, ischemic dilated cardiomyopathy.
TABLE 7. Continued

<table>
<thead>
<tr>
<th>Contractile response (mg)</th>
<th>Adenylate cyclase stimulation ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isoproterenol/NaF</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>Ca²⁺</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>...</td>
<td>...</td>
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<tr>
<td>...</td>
<td>...</td>
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<tr>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>1,602±299 (n=19)</td>
<td>1,003±224 (n=18)</td>
</tr>
<tr>
<td>1,390±141 (n=49)</td>
<td>1,165±112 (n=49)</td>
</tr>
<tr>
<td>1,001±83* (n=55)</td>
<td>1,171±94 (n=53)</td>
</tr>
</tbody>
</table>

As can be seen, the only significant correlation is between β₁-receptors and tissue norepinephrine in the IDC group. By polynomial (second-degree) regression analysis of β₁-receptor density versus tissue norepinephrine, the respective r values for Figures 4A–4D are 0.55 (p=0.0001), 0.16 (p=NS), 0.29 (p=NS), and 0.18 (p=NS).

Discussion

In investigations of adrenergic efferent systems in the failing heart, IDC and ISCDC are often grouped together,⁵,¹⁰ but it is unclear if this is justified. In an earlier muscle bath investigation of β-agonist–mediated contractile responses in end-stage failing hearts,¹⁰ we observed no obvious difference in isoproterenol responsiveness between preparations from ISCDC and IDC ventricles, but β-receptors were not measured, the sample size was relatively small, and the patient material was not stringently controlled for age or prior exposure to agents that might alter β-receptor function. Given the obvious differences in etiology, the potential exists for quantitative or qualitative differences in important physiopathological components of these two types of heart muscle disease. Such differences could account for the apparent disparity in degree of response of these two types of dilated cardiomyopathy to therapeutic intervention with β-blocking agents.²³

The data presented in the present study indicate that there are differences in the regulatory changes that occur in the β-receptor–effector mechanisms in these two types of dilated cardiomyopathy. Compared with IDC, ISCDC is characterized by less β-adrenergic receptor downregulation in both left and right ventricles, decreased coupling of β-adrenergic receptors mediating a contractile response in right ventricular tissue, and decreased coupling of β-adrenergic receptors mediating adenylate cyclase stimulation in left ventricular tissue.

FIGURE 3. Bar graph of changes in β-adrenergic receptors versus changes in muscle contraction in age- and pulmonary artery pressure (PAP)–restricted data in right ventricles (RVs) only. Data are expressed as percent of values in age-restricted nonfailing right ventricular controls (see Table 7). ISO, maximum net stimulation of right ventricular trabecular muscle contraction by isoproterenol; CA, maximum right ventricular trabecular stimulation by calcium; ISO/CA, ratio of stimulation by isoproterenol to stimulation by calcium; IDC, idiopathic dilated cardiomyopathy; ISCDC, ischemic dilated cardiomyopathy; B TOT, total β-receptor density; B-1, β₁-receptor density; B-2, β₂-receptor density; CK, creatine kinase; ISO, isoproterenol; CA, calcium.
The degrees of $\beta$-adrenergic receptor downregulation were different in ISCDC and IDC for both left and right ventricles. In the unrestricted analysis, $\beta$-receptor density in IDC (group B) ventricles was reduced by respective amounts in left and right ventricles of 43% ($p<0.05$ versus group A or C) and 51% ($p<0.05$ versus group A or C) compared with 30% ($p<0.05$ versus group A or B) and 33% ($p<0.05$ versus group A or B) in ISCDC (group C) ventricles. The behavior of total $\beta$-receptor density in ISCDC and IDC was a result of downregulation in the $\beta_1$-receptor population by respective amounts in left, right, and left plus right ventricles of 52%, 65%, and 56% in IDC compared with 38%, 47%, and 43% in ISCDC.

Furthermore, the difference in total $\beta$- and $\beta_1$-receptor densities in ISCDC versus IDC persisted in a restricted analysis that adjusted the ages in all three groups to 50–52 years and persisted after a second restricted analysis (for age and pulmonary artery pressure) that resulted in identical ages and hemodynamics in the ISCDC and IDC groups. In the age- and pulmonary artery pressure–restricted analysis, $\beta$-receptor density was 20% higher in left ventricles ($p<0.10$), 28% higher in right ventricles ($p<0.05$), and 24% higher in the combined left-plus-right ventricles group ($p<0.01$) of ISCDC compared with IDC. Similarly, $\beta_1$-receptor density was 33% higher in ISCDC left ventricles ($p<0.05$), right ventricles ($p<0.10$), or left plus right ventricles ($p<0.05$) compared with IDC. Therefore, with the greater statistical power of the combined (left plus right ventricles) group, both total $\beta$- and $\beta_1$-receptor densities were significantly higher in ISCDC than in IDC in the age-and pulmonary artery pressure–restricted analyses. Thus, the lesser amount of $\beta$-receptor downregulation exhibited by both ventricles of failing ISCDC hearts was not explained by age or differences in the degree of cardiac dysfunction in the two cardiomyopathy groups.

Despite having greater total $\beta$- and $\beta_1$-receptor densities, ISCDC left ventricles exhibited greater
blunting of β-agonist–mediated adenylate cyclase stimulation. In the unrestricted analysis, the nonselective β-agonist isoproterenol and the selective β2-agonist zinterol both resulted in lesser degrees of adenylate cyclase stimulation in ISCDC left ventricles than in IDC left ventricles; a statistically marginal but quantitatively similar pattern was noted for β-agonist stimulation in the age- and pulmonary artery pressure–restricted analyses. Also, in the age- and pulmonary artery pressure–restricted analyses, stimulation of adenylate cyclase by NaF and forskolin was diminished in ISCDC left ventricles relative to IDC left ventricles, whereas Mn2+ stimulation was not different in the two groups. Finally, isoproterenol stimulation was lower in ISCDC left ventricles than in ISCDC right ventricles, whereas isoproterenol stimulation in IDC left ventricles was higher than in IDC right ventricles. These data indicate that β-adrenergic receptors mediating adenylate cyclase stimulation in particular fractions of ISCDC left ventricles are more uncoupled from functional response than cyclase coupled β-receptors in IDC left ventricles. Furthermore, based on the NaF and Mn2+ data, this uncoupling probably involves abnormal G protein (G1 or G2) function rather than an abnormality in the catalytic unit of adenylate cyclase.

Despite the higher (relative to IDC) total β-receptor density in ISCDC right ventricles in the age- and pulmonary artery pressure–restricted analyses, the contractile response to the nonselective β-agonist isoproterenol was significantly lower in isolated preparations of ISCDC right ventricles. These data indicate that relative to β-receptors in IDC right ventricles, β-receptors in ISCDC right ventricles are moderately uncoupled from mechanical response. Unlike in left ventricles, right ventricular adenylate cyclase responses to isoproterenol, zinterol, and NaF were not reduced below levels in IDC right ventricles, with the ratios of NaF to isoproterenol and zinterol being reduced below nonfailing control values to a similar extent in ISCDC and IDC. However, the response to Gpp(NH)p was reduced to a greater extent in ISCDC right ventricles compared with IDC on all analyses. This suggests that the abnormality in β-agonist mechanical coupling in ISCDC right ventricles is related to abnormal G protein function but that it is different from the apparent G protein functional abnormality in ISCDC left ventricles.

The two kinds of functional responses measured in this study—positive inotropy and adenylate cyclase stimulation—are β-receptor subtype selective when mediated by a nonselective agonist such as isoproterenol. That is, the contractile response in isolated human tissue is mediated by β-receptor subtypes in direct proportion to their relative percentages such that stimulation by a nonselective agonist will primarily reflect stimulation of the majority β1-receptor. On the other hand, the tighter coupling of β2-receptors to adenylate cyclase in particular fractions of human ventricular myocardium results in stimulation by isoproterenol being mostly β2-receptor mediated, whereas zinterol stimulation is entirely β2-receptor mediated. This means that the functionality of β1-receptors (as assessed by mechanical response) was assessed only in right ventricular preparations, whereas the functionality of β2-receptors (adenylate cyclase stimulation by either isoproterenol or the β2-selective compound zinterol) was assessed in both left and right ventricles. Based on a comparison of receptor subtype density to functional response, the data are consistent with ISCDC right ventricular β2-receptors and left ventricular β2-receptors being relatively uncoupled from functional response compared with respective receptor subtypes in IDC right and left ventricles. Because β1-receptor density was not significantly different in ISCDC and IDC left and right ventricles, the lack of a difference in isoproterenol or zinterol stimulation of adenylate cyclase in ISCDC versus IDC right ventricles means that right ventricular β2-receptors did not exhibit a different coupling state in these two kinds of heart muscle disease. However, because zinterol-to-NaF ratios were reduced in all chambers of both IDC and ISCDC hearts while β2-receptors exhibited no change in density in any group, β2-receptors were mildly uncoupled from functional response in all failing chambers as previously reported in IDC. IDC left ventricular β2-receptors were therefore mildly uncoupled from functional response, whereas ISCDC β2-receptors were moderately uncoupled.

Because a β1-selective measurement of receptor pathway function was not performed in left ventricular preparations, the mechanical response coupling status of the less severely (relative to IDC) downregulated ISCDC left ventricular β1-receptor population was not addressed. Because the majority of adenylate cyclase stimulation in response to isoproterenol is β2-receptor mediated in preparations of human left ventricular myocardium, the greater blunting of isoproterenol responses in left ventricles from ISCDC versus IDC ventricles is not good evidence for uncoupling of left ventricular β2-receptors in ISCDC. However, because left ventricular β1-receptor density behaved quite similarly to right ventricular density in both ISCDC and IDC and isoproterenol stimulation of adenylate cyclase was more blunted in ISCDC, it is possible that the β2-receptor in ISCDC left ventricles also exhibited a relatively greater degree of uncoupling.

The reasons for the greater degree of uncoupling of ISCDC β-adrenergic receptors from functional response are not entirely clear. In left ventricles, two measurements of adenylate cyclase that are responsive to G protein function—NaF25 and forskolin stimulation of adenylate cyclase—were more blunted in ISCDC ventricles relative to preparations of IDC left ventricles or ISCDC right ventricles. On the other hand, the degree of stimulation by Gpp(NH)p in left ventricles was blunted to the same degree in both types of heart muscle disease, and substrates for pertussis (αG) or cholera (αG) toxin–catalyzed ADP ribosylation were not different in the two groups. αG, exhibited similar increases in both kinds of cardiomyopathy, whereas αG, was not different.
from nonfailing controls. Taken together, the adenylyl cyclase data in ISCDC left ventricles suggest a previously undescribed abnormality of G protein function that cannot be detected by ADP ribosylation experiments. Because F– can activate either Gs or Gt, and forskolin stimulation may be affected by either Gs or Gt, it is unclear where such an abnormality resides.

The nature of the abnormality in mechanical coupling of B-adrenergic receptors in ISCDC right ventricles is also unclear, but evidence from differential adenylyl cyclase responses to Gpp(NH)p suggests that it also involves differences in G protein function. Although B-agonist-mediated stimulation of adenylyl cyclase was not different in ISCDC versus IDC right ventricles, an abnormality of B1-receptor-mediated adenylyl cyclase stimulation would not have been detected by these B2-receptor probes, and B1-receptors were the apparent uncoupled subtype. There are numerous possible explanations for this previously undescribed abnormality of B1-receptor function in the failing human heart, including decreased protein kinase A activity, a defect at the level of cAMP-independent B1-receptor coupling to Ca2+ channels, receptor sequestration, and receptor phosphorylation. Because adenylyl cyclase responsiveness and the contractile response to Ca2+ were not altered in ISCDC right ventricles, it would appear that the observed B-receptor coupling abnormality in ISCDC does not involve the catalytic unit of adenylyl cyclase per se or an intrinsic property of the contractile elements.

Unlike in a recent report by Bohm et al., we observed an increase in pertussis toxin–catalyzed ADP ribosylation in preparations derived from ISCDC left ventricles and a decrease in Gpp(NH)p stimulation of adenylyl cyclase in ISCDC ventricles. The increased pertussis toxin–catalyzed ADP ribosylation in ISCDC left ventricles was of equal magnitude to that in IDC left ventricles, as was the decrease in Gpp(NH)p stimulation of adenylyl cyclase. The reasons for the difference between our findings and those of Bohm et al. are not entirely clear. However, it should be noted that the relatively fewer (10 ISCDC hearts and only five ISCDC left ventricles used for ADP ribosylation or adenylyl cyclase measurements) number of patients in Bohm et al.’s report had much better left ventricular function (ejection fraction, 29.6% versus 16.5% in the present study) than the ISCDC group in our study, and Bohm et al.’s ISCDC group also had better left ventricular function than their IDC group (ejection fraction, 20.8%). Thus, the ISCDC and IDC patients studied by Bohm et al. did not have comparable degrees of global left ventricular dysfunction, and cardiac function in their ISCDC group was less severely impaired than that in the ISCDC hearts used in our study. In addition, the statistical power of the data reported by Bohm et al. is limited by the presence of only three nonfailing controls.

One possible explanation for at least some of the observed differences in B-receptor downregulation in these two kinds of dilated cardiomyopathy is that in IDC a greater amount of adrenergic activation occurs, which leads to greater downregulation of B1-receptors, more catecholamine-mediated cardiotoxicity, and a better therapeutic response to B-blocking agents. Although tissue norepinephrine depletion, an index of adrenergic activation, and levels of the adrenergic cotransmitter neuropeptide Y were similar in ISCDC versus IDC, myocardial infarction may lead to sympathetic denervation in noninfarcted muscle. Therefore, partial depletion of neurotransmitters could have occurred in the noninfarcted regions of ISCDC ventricles as a result of denervation rather than as a result of exhaustion of neuronal neurotransmitter stores; such denervation would decrease both adrenergic drive and neurotransmitter levels. Support for denervation contributing to adrenergic neurotransmitter depletion in ISCDC ventricles was found in the lack of a relation between B1-receptor density and tissue norepinephrine level in group C, despite a highly significant correlation between these two parameters in group B. However, direct measurements of adrenergic drive and B-adrenergic receptors in patients with ISCDC versus those with IDC will be required to confirm the hypothesis that the latter is characterized by a greater degree of adrenergic drive for a given amount of myocardial dysfunction.

Previous studies in model systems have shown that exposure to B-agonists can both uncouple and downregulate B-adrenergic receptors. Potential causes for agonist-mediated B-receptor uncoupling include increased activity of Gs, decreased activity of Gt, and decreased activity of the catalytic subunit of adenylyl cyclase. In the failing human heart, adrenergic drive–mediated downregulation and uncoupling of B-adrenergic receptors may be inferred from investigations demonstrating that B-blockade induced upregulation and improved coupling of B-adrenergic receptors. When both types of subsensitivity phenomena are measured concurrently in model systems, uncoupling usually occurs first or at a lower dose of agonist, followed by downregulation. Based on these observations, it might be expected that higher degrees of adrenergic drive in heart failure would produce both receptor downregulation and uncoupling, whereas lesser degrees of adrenergic stimulation might produce uncoupling and either no or a lesser degree of receptor downregulation. The hypothesis of higher levels of adrenergic drive in IDC versus ISCDC as the potential explanation for the observed differences in B-receptor content and function would therefore not explain the greater degree of B-receptor uncoupling observed in group C. However, previous studies in model systems have shown that ischemia can produce myocardial B-receptor uncoupling and decreases in F– stimulation of adenylyl cyclase. Because the majority of the ISCDC hearts had severe, three-vessel coronary artery disease that might have
compromised the delivery of blood flow to either the left or right ventricle, the greater degree of uncoupling noted in ISCDC ventricles could have been a result of a recent or remote ischemic insult. However, because functional ischemia was not documented in this investigation, such an explanation for β-receptor uncoupling is only speculative.

Mn2+ stimulation of adenylate cyclase was mildly (by 34%) decreased in IDC right ventricles but not in IDC left ventricles or ISCDC ventricles. Mn2+ is thought to be a selective probe for adenylate cyclase catalytic unit (C) activity49 because unlike forskolin,26-28 Mn2+ activation of C is not affected by the presence of G proteins.49 The Mn2+ data suggest that IDC right ventricles have a mild abnormality of C, which is not evident on F− activation of G1 C. This defect in C may be related to pressure overload, because it is present to an even greater extent in right ventricles removed from patients with primary pulmonary hypertension.42 However, based on a comparison of the degree of downregulation of β-adrenergic receptors (by 51%) with the decrease in isoproterenol stimulation of muscle contraction (by 37% in the unrestrained analysis) in IDC right ventricles, it does not appear that the Mn2+-detectable decrease in C activity was transmitted to receptor uncoupling. That is, the abnormality does not manifest itself either in stimulation of adenylate cyclase by NaF or in a reduction in β-agonist–mediated muscle contraction beyond what would be expected from receptor downregulation.

Creatine kinase levels were higher by 20–30% in organ donor controls than in either cardiomyopathy group. In previous studies of nonfailing versus failing human heart, we have not found a significant difference in creatine kinase levels, although failing hearts tended to have slightly lower values.1,3,4,9,15 The reason for the larger difference between nonfailing and failing tissue noted in this study is that nonfailing controls were obtained exclusively from organ donors, whereas in previous studies left ventricles from patients with primary pulmonary hypertension were included in the control nonfailing population.1,3,4,9,15 Organ donors, perhaps because of hypovolemia and hypernatremia resulting from diabetes insipidus, have higher myocardial creatine kinase levels than primary pulmonary hypertension left ventricles, with values that are not different from those of patients with IDC or ISCDC (13 primary pulmonary hypertension left ventricles, creatine kinase=922±108 ng/g; p=NS versus ISCDC or IDC data in Table 3, unpublished data). In addition, creatine kinase levels were no longer different among the three groups after correction for age; that is, it was the younger donors who were contributing the most to the higher creatine kinase levels in group A. We conclude that the amount of functioning myocardium as assessed by tissue creatine kinase activity per gram of wet weight is not compromised in IDC or in the noninfarcted areas of ISCDC ventricles. Furthermore, the creatine kinase levels were nearly identical in tissue aliquots taken from the noninfarcted areas of ISCDC ventricles and IDC ventricles. These data plus the similarity of the calcium-mediated contractile responses in right ventricular preparations taken from ISCDC and IDC ventricles indicate that increased tissue fibrosis in samples taken from ISCDC ventricles was not the explanation for the pharmacological differences noted between ISCDC and IDC.

As summarized in Table 8, potentially important differences exist in the degree and profile of regulatory changes that occur in the β-adrenergic neuroeffector system in ISCDC versus IDC. Compared with IDC ventricles, ISCDC ventricles exhibit less marked total β- and β1-receptor downregulation while exhibiting a greater degree of uncoupling of left ventricular β-receptors and right ventricular β1-receptors from mechanical response. The coupling abnormality in ISCDC left ventricles probably arises from an abnormality of G1 or a relatively increased functional activity of G1 that is not detectable by toxin-catalyzed ADP ribosylation. The differences in behavior of various components of the β-adrenergic receptor–G protein–adenylate cyclase complex in ISCDC versus IDC probably arise from differences in the processes that produce the underlying tissue damage in these two types of heart muscle disease. This in turn creates the potential for a heterogeneous response to pharmacological interventions designed to alter the natural history of dilated cardiomyopathies. Although various types of end-stage heart muscle disease may exhibit common features, there may be important pathophysiologica differences.

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>IDC</th>
<th>ISCDC</th>
</tr>
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<tbody>
<tr>
<td>Neurotransmitter depletion</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Downregulation of β1-adrenergic receptors</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Uncoupling of β2-adrenergic receptors from adenylate cyclase</td>
<td>+</td>
<td>+ (RV)</td>
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<tr>
<td>F−, forskolin-sensitive G protein functional abnormality in adenylate cyclase stimulation</td>
<td>+</td>
<td>+ (LV)</td>
</tr>
<tr>
<td>Increased activity of G1</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Uncoupling of β2-adrenergic receptors from mechanical response</td>
<td>−</td>
<td>+ +</td>
</tr>
<tr>
<td>Gpp(NH)p sensitive G protein functional abnormality in adenylate cyclase stimulation</td>
<td>+</td>
<td>+ (LV)</td>
</tr>
<tr>
<td>Decreased catalytic subunit activity</td>
<td>+ (RV only)</td>
<td>−</td>
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</table>

IDC, idiopathic dilated cardiomyopathy; ISCDC, ischemic dilated cardiomyopathy; RV, right ventricles; LV, left ventricles. +, Mild; ++, moderate; ++++, marked.
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References
35. Sandoval AB, Gilbert EM, Rose CP, Bristow MR: Cardiac norepinephrine uptake and release is decreased in dilated cardiomyopathy (abstract). *Circulation* 1989;80(suppl IV):IV-393


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Differences in beta-adrenergic neuroeffector mechanisms in ischemic versus idiopathic dilated cardiomyopathy.
M R Bristow, F L Anderson, J D Port, L Skerl, R E Hershberger, P Larrabee, J B O'Connell, D G Renlund, K Volkman and J Murray

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