**β₁- and β₂-Adrenergic-Receptor Subpopulations in Nonfailing and Failing Human Ventricular Myocardium: Coupling of Both Receptor Subtypes to Muscle Contraction and Selective β₁-Receptor Down-Regulation in Heart Failure**

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We used radioligand binding techniques and measurement of β-agonist-mediated positive inotropic responses in isolated cardiac tissue to examine β-adrenergic-receptor subpopulations in nonfailing and failing human left and right ventricular myocardium. In tissue derived from 48 human hearts the receptor subtypes identified in nonfailing ventricle by radioligand binding were β₁ (77%) and β₂ (23%), with no evidence of an "atypical" β-adrenergic receptor. In failing left ventricle the β₁:β₂ ratio was markedly different, i.e., 60:38. This decrease in the β₁ proportion and increase in the β₂ proportion in the failing ventricles were due to a 62%, "selective" down-regulation of the β₁ subpopulation, with little or no change in β₂ receptors. In muscle bath experiments in isolated trabeculae derived from nonfailing and failing right ventricles, both β₁- and β₂-adrenergic receptors were coupled to a positive inotropic response. In nonfailing myocardium, β₁ responses predominated, as the selective β₁ agonist denopamine produced a response that was 66% of the total contractile response of isoproterenol. In heart failure the β₁ component was markedly decreased, while the β₂ component was not significantly diminished. Moreover, in heart failure the β₂ component increased in prominence, as the contractile response to the selective β₂ agonist zinterol increased from a minority (39%) to a majority (60%) of the total response generated by isoproterenol. We conclude that failing human ventricular myocardium contains a relatively high proportion of β₁ receptors, due to selective down-regulation of β₁ receptors. As a result, in the failing human heart the β₂-receptor subpopulation is a relatively important mediator of inotropic support in response to nonselective β-agonist stimulation and is available for inotropic stimulation by selective β₂ agonists. (Circulation Research 1986;59:297–309)

Previous studies using radioligand binding techniques have shown that human myocardial tissue contains a relatively high proportion of the β₁-adrenergic-receptor subtype.¹⁻⁴ In these studies the percent of β₁ receptors has ranged from 14 to 40% in ventricular myocardium and from 20 to 55% in atrial tissue. These findings are in contrast to data from other types of mammalian myocardium, where β₁-adrenergic receptors have generally not been found in ventricular myocardium⁷⁻¹⁰ and only small percentages of β₂ receptors have been identified in atrial tissue of some mammalian laboratory animal species.¹¹ Limited tissue bath data in isolated human ventricular myocardium suggest that the β₂-adrenergic receptor is coupled to a positive inotropic response.⁶,¹²,¹³ Such information is essential for establishing a cardiomyotropic origin of β₂-adrenergic receptors identified biochemically because cardiac tissue contains a number of nonmyocytic cell types that would be expected to contain β₂ receptors. However, since no quantitative information on the relative importance of the myocardial β₂ receptor population was provided in these previous studies it is uncertain what physiologic or pathophysiologic purpose is subserved by this class of β₂-adrenergic receptors.

Heart failure is associated with major alterations in the β₂-adrenergic receptor population in human ventricular myocardium.¹⁴⁻¹⁶ Failing human left ventricle has a decreased total population of β receptors, which confers subsensitivity to β-receptor-mediated mechanical events.¹⁴⁻¹⁶ Previous studies on the behavior of β-adrenergic receptors in heart failure have examined...
only the total β-receptor population and have not reported information on β₁ and β₂ subtypes. Because of these issues we thought it important to examine the effect of heart failure on ventricular myocardial β₁- and β₂-adrenergic receptors in the human heart. The investigation was designed to investigate further the questions of whether the cardiac β₁-receptor subpopulation is present on myocardial cells and if this subtype is capable of mediating a mechanical response, as well as to determine the relative importance of the β₁- and β₂-receptor subpopulations in nonfailing and failing human ventricular myocardium.

Materials and Methods

Tissue Procurement

Human hearts were obtained from 31 subjects with severe end-stage biventricular failure (BVF) due to idiopathic dilated cardiomyopathy, 6 subjects with isolated right ventricular failure due to primary pulmonary hypertension (PPH), and 11 would-be cardiac transplant donors with no evidence of cardiac dysfunction. Of the 31 hearts from BVF subjects, 19 were used only for tissue bath studies, 9 were used exclusively for biochemical measurements, and 3 were used for both. The 6 hearts from PPH subjects were used for biochemical studies only. Of the 11 hearts from would-be donors, 5 were used exclusively for tissue bath experiments, 4 were used only for biochemical studies, and 2 were used for both.

In transplant recipients and 3 “on-site” donors the hearts were excised and immediately immersed in ice-cold, oxygenated physiologic salt solution. In these hearts the elapsed time between cardiac excision, weighing, and homogenization was < 30 minutes in all cases. In 3 distantly procured hearts the time between cardiac excision and homogenization was under 2.5 hours, with the hearts harvested as previously described and maintained under hypothermic conditions (4°C) until homogenization.

In BVF hearts used for muscle bath studies the average cardiac catheterization values obtained within 8 weeks of transplant (± SEM) were: right atrial mean pressure, 9.3 ± 1.4 mm Hg; pulmonary wedge mean pressure, 25.8 ± 1.9 mm Hg; cardiac index, 2.2 ± 0.3 L/minute per m². In BVF hearts used for biochemical studies the right atrial mean pressure was 13.9 ± 3.1 mm Hg, pulmonary wedge mean pressure 26.7 ± 2.7 mm Hg, and the cardiac index 2.1 ± 0.3 L/minute per m². The 6 PPH patients were considered to have relatively normal left ventricular function and severe, isolated right ventricular failure on the basis of catheterization and echocardiographic data. In this group the pulmonary artery systolic pressure averaged 86 ± 9 mm Hg, the mean pulmonary artery pressure was 56 ± 5 mm Hg, the mean pulmonary wedge pressure was 6.0 ± 2.2 mm Hg, and the mean cardiac index was 1.8 ± 0.2 L/minute per m². The PPH group echocardiographically determined left ventricular end diastolic dimension and wall motion was normal in 5 subjects in whom echocardiography was performed.

The 6 would-be donors were normally functioning young adults aged 17–40 years who had suffered trauma-related brain death prior to organ procurement. These individuals had been maintained on respirators for 2–5 days prior to organ harvest. These 6 hearts would have been used for donor grafts except for late-developing complications in recipients (n = 4), no suitable recipient match (n = 2), and the presence of an atrial laceration in one.

No recipient or donor heart had been exposed to β-agonists or antagonists within 48 hours of organ procurement. Medications in the BVF and PPH groups did not differ from previous reports and included diuretics, digoxin, and vasodilators. In the BVF groups explanted hearts used for tissue bath studies were from subjects averaging 37 ± 3 years in age (median 40), while in those BVF hearts used for biochemical studies the average age was 32 ± 4.1 (median 34). The PPH group averaged 34 ± 3 years, with a median of 34. The 11 donors averaged 26 ± 2.5 years with a median of 26; the median for donors used in muscle bath studies was 24, and for biochemical experiments 20. By ANOVA there was no significant difference in age among any of these groups. Pooling the BVF groups and comparing all subjects with BVF to the donor plus PPH groups (groups with nonfailing left ventricles) also yielded no significant difference in age (nonfailing 29 ± 2 years, BVF 36 ± 2 years, p = ns).

Twenty-four of the 31 BVF hearts were from male recipients, while 9 of the 11 donors were male. Four of the 6 PPH subjects were female.

Written permission for organ donation for clinical or research purposes or for heart or heart–lung transplantation was obtained in all cases. Hearts were obtained from the Stanford and Utah cardiac transplant programs.

Protocol

For tissue bath studies material was divided into nonfailing (Group A) donors and failing (Group B) recipients with BVF. For biochemical experiments left ventricular (LV) samples were divided into Group A, consisting of 6 nonfailing donor LV’s and 6 LV’s from PPH subjects, and Group B, 12 BVF left ventricles. Additionally, in biochemical studies right ventricular measurements were performed in 4 donor hearts, 4 BVF hearts, and 5 PPH hearts. The 4 nonfailing donor RV’s were assigned to Group A, while the 4 failing BVF and 5 failing PPH RV’s were assigned to Group B.

Contractile Response of Isolated Right Ventricular Trabeculae

The contractile response of isolated human cardiac preparations was assessed as previously described. Twenty-two failing right ventricles from hearts of BVF transplant recipients and 7 right ventricles from donor hearts had 8 trabeculae per heart removed, dissected to uniform size of 0.5–1.0 × 6.0–7.0 mm and mounted in a multichamber muscle bath. In 16 hearts (7 donor and
9 BVF) 2-4 trabeculae per pharmacologic subset were used to evaluate the contractile response to the nonselective β-agonist isoproterenol, the selective β₁-agonist denopamine (TA064)
and the selective β₁-agonist zinterol. Additionally, in 20 BVF hearts the effects of the selective β₂-antagonist betaxolol and the selective β₂-antagonist ICI 118,551 on the dose-response to isoproterenol or zinterol was assessed in isolated RV trabeculae. Antagonists were incubated with tissue for 1 hour prior to performing agonist dose-response curves. Baseline tension, bathing fluid, and stimulation characteristics were as previously described. Response was recorded as the net increase in milligrams tension, defined as measured tension minus baseline tension.

Biochemical Studies

A 5- to 6-g aliquot of left and right ventricular free wall was dissected free of epicardial fat, endocardial fibrosis, chordae, and papillary muscles and placed in ice-cold, 10 mM Tris, 1 mM EGTA buffer pH 8.0. The tissue was finely minced with scissors and homogenized with a Polytron (Binkmann Instruments, Westbury, N.Y.) using 3 consecutive bursts of 5 seconds each at full speed. A crude membrane fraction was made by extracting the contractile proteins in 0.5 M KCl and washing a 50,000 g pellet, as previously described. In 7 hearts (+) [125Iodocyanopindolol (ICYP) binding isotherms (see below) were measured in freshly prepared membranes. In the other 17 hearts membranes were resuspended in 250 mM sucrose, 50 mM Tris, 1 mM EGTA pH 7.5 buffer at a protein concentration of 5–10 mg/ml and stored at −80°C for up to 8 weeks until used. Membranes stored in this manner retained 96% of their β-receptor density as judged from a comparison of 7 LV preparations assayed immediately at the time of harvest to the same preparations assayed later after storage. Relative to the washed 50,000 g pellet of the homogenate, left ventricular membranes prepared in this manner had a 3.1 ± 0.6 fold enrichment in β-adrenergic receptor density (ICYP binding).

For receptor assays the total population of β-adrenergic receptors was radiolabeled with ICYP using previously described methods. Assays were conducted in 150 mM NaCl, 20 mM Tris, 1 mM ascorbate buffer pH 7.5 at 30°C. Incubation was for 120 minutes, by which time steady state was achieved. The protein concentration varied between 33 and 169 g/ml (average 123 ± 12 g/ml). Maximum binding (B_max) and dissociation constant (K_d) were determined by nonlinear least-squares fit of the specific binding curve, as previously described. Seven increasing concentrations of ICYP between 3.125 and 150 or 200 pM in the presence and absence of 1 M (−) propranolol were used to construct the specific binding isotherm. In all assays performed, specific binding of ICYP was ≥ 90% of total binding at low (< 6 pM) radioligand concentrations. In calculating binding parameters ICYP was assumed to undergo molecular decomposi-

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ition into non-β-receptor active products in direct relation to radioactive decay.

β₁ versus β₂-adrenergic receptor subtypes were identified by computer modeling of 17–19 point betaxolol–ICYP competition curves performed in each preparation. Additionally, in 9 LV and 4 RV Group A preparations 17–19 point ICI 118,551–ICYP competition curves were also performed, and in 12 LV preparations (±) propranolol–ICYP competition curves were performed. Assay conditions for competition curves were identical to those for ICYP specific binding isotherms, except that the assay buffer was 135 mM NaCl, 10 mM MgCl₂, 1 mM ascorbate pH 7.5. The ICYP concentration was 50 pM, and the receptor concentration ranged from 2.27 to 9.78 pM, with an average of 4.83 ± 0.51 pM. Competition curves were analyzed by two different techniques that are described in an appendix.

In muscle bath experiments dissociation constants (K_d values) of β-antagonists were derived from the equation $K_d = \frac{(\text{dose ratio} - 1)}{\text{dose ratio} - 1}$.  

Adenylate cyclase in crude membranes and homogenates was measured as previously described, while 5’ nucleotidase activity was measured by the method of Ipata.  

Creatine kinase activity in soluble fractions of spun homogenates was determined as previously described. Proteins were measured by the Peterson modification of the Lowry technique.

Statistical Analysis

For comparison of binding parameters between Group A and Group B Student’s t test was used, with p < .05 in a two-tailed distribution taken as statistical significance. Dose-response curves were analyzed using the multivariate analysis of variance analog of the univariate repeated measures analysis of variance described by Potoff and Roy, Khatri, and Grizzle and Allen and summarized by Morrison. This analysis allows tests of the difference in polynomial trends in the dose-response curves between treatment groups. The APL matrix calculation language run on a VAX computer (Digital Equipment Corp., Boston, Mass.) was used to obtain matrices necessary for the analysis (orthogonal polynomials via Cholsky decomposition, and the product of those polynomials and the original data set), and the analysis was performed using the general multivariate linear model program, BMD11V run on an IBM PCXT computer (IBM computers, Boca Raton, Fla.). To detect potential differences in dose–response curves, this analysis was applied to points between the ED₅₀ and ED₉₀ of the mean values of the curves without added antagonist (control curve), and a comparison in the response at these points was made between the control curves and the curves performed in the presence of antagonist. A p (F) < .05 was taken as statistical significance. For comparison of data among > 2 groups, ANOVA and the Bonferroni t test was employed.
Betaxolol was obtained from Synthelabo, (L.E.R.S.) Paris, France. ICI 118,551 was a gift from Imperial Chemical Industries, Cheshire, England. Propranolol was obtained from Ayerst Laboratories, New York, N.Y. Zinterol was obtained from Bristol Myers, Evansville, Ind., while denopamine (TA064) was obtained from Marion Laboratories, Kansas City, Mo. ICYP was purchased from Amersham, Arlington Heights, Ill. All other chemicals were from standard commercial suppliers.

Results

β-Adrenergic Receptor Density Measurements

[125I] iodocyanopindolol (ICYP) specific binding isotherm data are given in Figure 1 and Table 1. Figure 1 gives representative binding isotherms for ICYP in preparations derived from nonfailing (Group A) and failing (Group B) left ventricles. Plots of bound/free vs. bound are shown in the figure insets. ICYP demonstrated saturable, highly specific binding to a single class of sites, as shown by linear plots of bound/free...
Identification and Quantification of $\beta_1$ and $\beta_2$ Populations

**Group A (Nonfailing Ventricle): Left Ventricle.** In preparations derived from nonfailing left ventricle, a selective $\beta_1$ competitive antagonist yielded comparable values for proportion of $\beta_1$- and $\beta_2$-receptors ($p > .05$ for both). All but one preparation modeled for a 2-site fit, for both programs; this preparation modeled for 2 sites with betaxolol-ICYP. Betaxolol competition curves yielded a lower coefficient of variation for receptor subtype proportions (Table 4) and so this ligand was chosen for routine use.

Table 5 gives the proportion of $\beta_1$- and $\beta_2$-receptors and $K_D$ values for betaxolol–ICYP competition curves in left ventricular myocardium for 6 donor versus 6 PPH hearts that comprised Group A. There are no significant differences in total $\beta$-adrenergic receptor density, $\beta_1$ or $\beta_2$ proportion or $K_D$ values in these two subgroups. Also, there were no significant differences in total $\beta$-receptor density or proportion of receptor subtypes comparing the 6 male and 6 female subjects.

Propranolol–ICYP competition curves were performed in 3 normal left ventricles. All 3 gave best fits for 1 site, for both programs. The mean $K_D$ value (MLAB) was 3.76 nM, with a mean slope factor of 0.98.

**Group B (Failing Ventricle): Left Ventricle.** Right ventricular values from ICYP–betaxolol competition curves in 4 donor hearts were not significantly different from the respective values in donor LV: fraction $\beta_1$; $\beta_2$ (MLAB), .765 ± .040; .232 ± .033; $\beta_1$; $\beta_2$ receptor density calculated from ICYP $B_{max}$, 61.7 ± 11.9:17.2 ± 2.1 fmol/mg.

ICYP–ICI 118,551 competition curves also yielded data similar to donor LV: fraction $\beta_1$; $\beta_2$ (MLAB), .795 ± .039: .174 ± .042; $\beta_1$; $\beta_2$ receptor density calculated from ICYP $B_{max}$, 61.0 ± 13.3:14.9 ± 4.1.

**Group B (Failing Ventricle): Right Ventricle.** Betaxolol–ICYP competition curves were performed in 12 BVF left ventricles, and in 8 of them a propranolol–ICYP competition curve was also performed. A representative set of betaxolol/propranolol–ICYP competition curves is given in Figure 3. The best fit for the propranolol curve shown in Figure 3 is for 1-site, with a $K_D$ (MLAB) of 1.57 nM (MLAB). The best fit for the betaxolol curve is for 2 sites with proportions of high- and low-affinity receptors of 0.566 and 0.414 respectively, and $K_D$ values of 4.21 and 112 nM. Propranolol–ICYP competition curves performed in 8 other

<table>
<thead>
<tr>
<th>Fraction, assay</th>
<th>Nonfailing</th>
<th>Failing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Membrane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. 5' nucleotidase (nmol/min/mg)</td>
<td>10.5 ± 1.2</td>
<td>14.3 ± 2.9</td>
</tr>
<tr>
<td>b. Adenylate cyclase (pmol/min/mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1) Basal</td>
<td>17.0 ± 2.8</td>
<td>13.7 ± 1.6</td>
</tr>
<tr>
<td>2) 10 mM fluoride</td>
<td>51.7 ± 8.9</td>
<td>59.6 ± 8.0</td>
</tr>
<tr>
<td>3) 0.1 mM forskolin</td>
<td>258 ± 46</td>
<td>258 ± 58</td>
</tr>
<tr>
<td>2. Soluble-creatine kinase (IU/g wet wt.)</td>
<td>1063 ± 83</td>
<td>909 ± 70</td>
</tr>
</tbody>
</table>

None of the differences were statistically significant.

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failing hearts all yielded a 1-site fit for both programs, with an average $K_D$ (MLAB) ± SEM for all 9 curves of 2.73 ± 0.55 nM, and an average slope factor of 1.01 ± .05.

Betaxolol–ICYP competition curves yielded a 2-site fit in all 12 failing LV’s, for both programs. A summary of these data is given in Table 3. Compared to Group A, the slope factor in failing group B LV’s is lower, the proportion of $\beta_1$ receptors is lower and the proportion of $\beta_2$ receptors is higher in failing left ventricle. There was no significant difference in the high- or low-affinity $K_i$ in the two groups. For mean data in failing LV’s given in Table 3 the two types of computer modeling yielded similar relative proportions of $\beta_1$ and $\beta_2$ receptors; .664 and .337 for LIGAND (both $p < .001$) vs. respective subgroup in A. and .600 and .386 for MLAB (both $p < .001$).

The concentrations of $\beta_1$ and $\beta_2$ receptors calculated from ICYP $B_{max}$ values in nonfailing (Group A) and failing (Group B) human left ventricular myocardium are given in Table 6. Compared to nonfailing chambers, failing human left ventricle has 61.3% fewer $\beta_1$-adrenergic receptors. On the other hand, $\beta_2$-adrenergic receptors in failing left ventricles were decreased by only 20.5%. In failing left ventricles the decrease in $\beta_1$-adrenergic receptor density was highly significant ($p < .001$), while the $\beta_2$-receptor measurement was not significantly decreased ($p > .10$).

**GROUP B (FAILING VENTRICLES): RIGHT VENTRICLE.** Values for $\beta$-receptor subpopulations in the 4 right ventricles from BVF hearts were similar to those in BVF RV and LV: fraction $\beta_1$; $\beta_2$ (MLAB), .592 ± .063: .396 ± .060; $\beta_1$; $\beta_2$ receptor density calculated from ICYP $B_{max}$, 2.14 ± 1.4: 14.8 ± 5.0. Because failing RV’s from BVF and PPH hearts did not differ, these nine hearts were pooled together in Table 6.

Betaxolol–ICYP competition curves were performed in 5 failing RV’s from PPH hearts, and the results were similar to BVF RV and LV: fraction $\beta_1$; $\beta_2$ (MLAB), .620 ± .060:.366 ± .053; $\beta_1$; $\beta_2$ receptor density calculated from ICYP $B_{max}$, 25.8 ± 3.9: 16.7 ± 5.0. Because failing RV’s from BVF and PPH hearts did not differ, these nine hearts were pooled together in Table 6.

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**Table 3. Data Summary: Betaxolol–ICYP Competition Curves in Nonfailing (Group A) and Failing (Group B) Human Left Ventricle.**

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Curve slope (Hill #)</th>
<th>Fraction $\beta_1$ (%)</th>
<th>Fraction $\beta_2$ (%)</th>
<th>$K_i$ (nM) MLAB</th>
<th>Fraction $\beta_1$ (%)</th>
<th>Fraction $\beta_2$ (%)</th>
<th>$K_i$ (nM) Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, nonfailing</td>
<td>0.74</td>
<td>0.773</td>
<td>0.228</td>
<td>5.01</td>
<td>210</td>
<td>0.809</td>
<td>0.193</td>
</tr>
<tr>
<td>(12)</td>
<td>±0.03</td>
<td>±0.028</td>
<td>±0.027</td>
<td>±1.27</td>
<td>±49</td>
<td>±0.025</td>
<td>±0.021</td>
</tr>
<tr>
<td>B, failing</td>
<td>0.64*</td>
<td>0.600†</td>
<td>0.386†</td>
<td>5.257</td>
<td>235‡</td>
<td>0.664†</td>
<td>0.337†</td>
</tr>
<tr>
<td>(12)</td>
<td>±0.01</td>
<td>±0.024</td>
<td>±0.021</td>
<td>±0.69</td>
<td>±64</td>
<td>±0.021</td>
<td>±0.021</td>
</tr>
</tbody>
</table>

* $p > .01$ vs. respective subgroup in A.
† $p < .0001$ vs. respective subgroup in A.
‡ $p > .10$ vs. respective subgroup in A.
Receptors to Muscle Contraction

Coupling off and f1 human ventricular trabeculae it produces a maximum response that is 40–50% of the isoproterenol maximum. The ED50 for the mean zinterol curve shown in Figure 4 is 2.05 × 10^-7 M, while the ED50 of the isoproterenol curve is 2.13 × 10^-7 M.

Figure 4 gives the antagonism of the isoproterenol response by 10^-7 M betaxolol. This concentration of betaxolol would be expected to occupy ≥90% of the high-affinity (β1) receptor sites, and <5% of the low affinity (β2) site. Betaxolol antagonized the effects of isoproterenol, as dose–response curves were significantly right shifted compared to the curve performed in the absence of antagonist [X intercept of points between ED10 and ED50 are significantly different (p < .05)]. Figure 4 also gives the effect of 10^-7 M betaxolol on the zinterol dose–response curve. In contrast to the effect of betaxolol on isoproterenol, the zinterol dose–response curve is not significantly altered (p > .05) by betaxolol. The K9 value calculated for betaxolol antagonism of isoproterenol from the mean curves shown in Figure 4 was 5.16 × 10^-4 M. The K9 values for betaxolol antagonism of isoproterenol calculated from individual muscle bath experiments was significantly greater than the high affinity K9 value calculated from betaxolol ICYP competition curves: (K9 ± SEM = 4.29 ± 2.73 × 10^-4 M, K9 (MLAB) 5.10 ± 0.56 × 10^-4; p < .05).

Figure 5 shows that the effect of zinterol is markedly antagonized by ICI 118,551, which produced a > ten-fold shift in the mean zinterol dose–response curve. Because the curve in the presence of antagonist was so right shifted and did not begin to ascend until a dose of 6 × 10^-5 M was reached it yielded a significantly different (p < .05) slope comparing points between the control curve ED50 and the ED50 when the curves were modeled to a first-degree polynomial. Additional evidence for a significant blockade of zinterol by ICI 118,551 was that individual points at zinterol concentr-

Comparison of Left Ventricle to Right Ventricle in Hearts with Similar Degree of Ventricular Function

Table 6 gives β-receptor data for failing and nonfailing LV and RV, with PPH LV included in the nonfailing group and PPH RV in the failing group. As can be seen in Table 6, there were no differences between total receptor density or subpopulation data in LV versus RV in either failing or nonfailing chambers. Moreover, differences between nonfailing and failing RV are quite similar to differences between nonfailing and failing LV.

If nonfailing LV and RV are grouped and compared to failing LV and RV (Table 6) the average decrease in total β receptor density by 51.2%, the decrease in β1 receptor density is by 61.9%, and the average decrease in β2 receptor density is by 18.9%. For combined LV and RV analysis the difference between nonfailing and failing total or β2 receptor density was highly significant (p < .001), while the β2 receptor density was not different (p > .10).

Coupling of β1 and β2 Receptors to Muscle Contraction

As nonmyocardial cell types extant in cardiac tissue may constitute a significant portion of membranes derived from mammalian heart, it is important to demonstrate that β2-adrenergic receptors in human ventricular myocardium are coupled to a mechanical response, or present on myocardial cells.

Figures 4 and 5 give muscle contraction data for the response of isolated right ventricular trabeculae to either isoproterenol or zinterol in the presence and absence of selective antagonists in preparations from failing right ventricles. In Figure 4 the full dose-response to isoproterenol can be compared to zinterol in RV trabeculae derived from 18 failing hearts. Zinterol is a partial agonist with selective β1 properties, and in human ventricular trabeculae it produces a maximum response that is 40–50% of the isoproterenol maximum. The ED50 for the mean zinterol curve shown in Figure 4 is 2.05 × 10^-7 M, while the ED50 of the isoproterenol curve is 2.13 × 10^-7 M.

Figure 4 gives the antagonism of the isoproterenol response by 10^-7 M betaxolol. This concentration of betaxolol would be expected to occupy ≥90% of the high-affinity (β1) receptor sites, and <5% of the low affinity (β2) site. Betaxolol antagonized the effects of isoproterenol, as dose–response curves were significantly right shifted compared to the curve performed in the absence of antagonist [X intercept of points between ED10 and ED50 are significantly different (p < .05)]. Figure 4 also gives the effect of 10^-7 M betaxolol on the zinterol dose–response curve. In contrast to the effect of betaxolol on isoproterenol, the zinterol dose–response curve is not significantly altered (p > .05) by betaxolol. The K9 value calculated for betaxolol antagonism of isoproterenol from the mean curves shown in Figure 4 was 5.16 × 10^-4 M. The K9 values for betaxolol antagonism of isoproterenol calculated from individual muscle bath experiments was significantly greater than the high affinity K9 value calculated from betaxolol ICYP competition curves: (K9 ± SEM = 4.29 ± 2.73 × 10^-4 M, K9 (MLAB) 5.10 ± 0.56 × 10^-4; p < .05).

Figure 5 shows that the effect of zinterol is markedly antagonized by ICI 118,551, which produced a > ten-fold shift in the mean zinterol dose–response curve. Because the curve in the presence of antagonist was so right shifted and did not begin to ascend until a dose of 6 × 10^-5 M was reached it yielded a significantly different (p < .05) slope comparing points between the control curve ED50 and the ED50 when the curves were modeled to a first-degree polynomial. Additional evidence for a significant blockade of zinterol by ICI 118,551 was that individual points at zinterol concentr-

Table 4. Comparison of Results for Betaxolol vs. ICI 118,551 Competition Curves in Nine Nonfailing Left Ventricles, MLAB Analysis ± SEM

<table>
<thead>
<tr>
<th>Group</th>
<th>Bmax (fmol/mg)</th>
<th>ICYP</th>
<th>Fraction</th>
<th>K1 (nM)</th>
<th>K2 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donors</td>
<td>81.7</td>
<td>± 9.5</td>
<td>± 0.94</td>
<td>0.204</td>
<td>4.46</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>94.2</td>
<td>± 0.73</td>
<td>± 0.03</td>
<td>0.029</td>
<td>± 3.35</td>
</tr>
<tr>
<td>PPH</td>
<td>± 11.2</td>
<td>± 1.57</td>
<td>± 0.04</td>
<td>0.251</td>
<td>± 0.02</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>66.7</td>
<td>± 1.12</td>
<td>± 0.046</td>
<td>0.298</td>
<td>± 3.56</td>
</tr>
</tbody>
</table>

Table 5. Comparison of Nonfailing Left Ventricles from Donor Hearts to Left Ventricles from Heart–Lung Transplant Recipients with Isolated RV Failure Due to Primary Pulmonary Hypertension (PPH). ICYP–Betaxolol Competition Curves, MLAB Analysis ± SEM

<table>
<thead>
<tr>
<th>Group</th>
<th>Bmax (fmol/mg)</th>
<th>ICYP</th>
<th>Fraction</th>
<th>K1 (nM)</th>
<th>K2 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donors</td>
<td>42.0</td>
<td>± 0.73</td>
<td>± 0.03</td>
<td>0.298</td>
<td>± 0.02</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>66.7</td>
<td>± 1.12</td>
<td>± 0.046</td>
<td>0.298</td>
<td>± 3.56</td>
</tr>
</tbody>
</table>
trations of $10^{-8}$ and $3 	imes 10^{-8}$ M were significantly different ($p < .05$). Because of the relatively small effect of zinterol on milligrams of tension it was not possible to derive reliable $K_B$ values for individual experiments, but the $K_B$ value derived from the mean curve shown in Figure 5 would be approximately 0.5 nM, which is similar to the high-affinity $K_B$ value derived from ICYP-ICI 118,551 competition curves (0.71–1.50 nM, Table 3).

**Effect of Nonselective and Selective $\beta$ Agonists on Maximum Tension**

In Figure 6 are given the maximum net tension responses of isolated RV trabeculae to the selective $\beta_1$ partial-agonist denopamine,20 the selective $\beta_2$ partial-agonist zinterol22,25 and the nonselective full $\beta$-agonist isoproterenol. In 7 nonfailing, Group A hearts isoproterenol produced a maximum response that was 2.5 times that of zinterol and 1.5 times greater than that of denopamine.

### Table 6. Comparison of Left Ventricle (LV) to Right Ventricle (RV) in Groups With Similar Degree of Function in Each Chamber (Nonfailing and Failing), Mean (MLAB Analysis) ± SEM

<table>
<thead>
<tr>
<th>Group</th>
<th>Saturation curves</th>
<th>Betaxolol-ICYP competition curves</th>
<th>Subpopulation receptor density (fmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total $\beta$ receptor density (fmol/mg)</td>
<td>Fractions $\beta_1$</td>
<td>Fractions $\beta_2$</td>
</tr>
<tr>
<td></td>
<td>ICYP $B_{max}$</td>
<td>$\beta_1$</td>
<td>$\beta_2$</td>
</tr>
<tr>
<td>A. (Nonfailing)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. LV</td>
<td>88.0 ± 7.3</td>
<td>±0.028 ±0.027</td>
<td>67.0 ± 4.5</td>
</tr>
<tr>
<td>n = 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. RV</td>
<td>78.8 ± 13.0</td>
<td>±0.040 ±0.033</td>
<td>61.7 ± 0.9</td>
</tr>
<tr>
<td>n = 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. LV + RV</td>
<td>85.7 ± 6.2</td>
<td>±0.023 ±0.021</td>
<td>65.6 ± 4.3</td>
</tr>
<tr>
<td>n = 16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. (Failing)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. LV</td>
<td>43.2* ± 3.4</td>
<td>±0.024 ±0.021</td>
<td>25.9* ± 2.4</td>
</tr>
<tr>
<td>n = 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. RV</td>
<td>40.0* ± 4.6</td>
<td>±0.041 ±0.037</td>
<td>23.8* ± 2.2</td>
</tr>
<tr>
<td>n = 9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. LV + RV</td>
<td>41.8* ± 2.7</td>
<td>±0.022 ±0.020</td>
<td>25.0* ± 1.6</td>
</tr>
<tr>
<td>n = 21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < .0001 vs LV + RV group A or LV group A.
†p .05 vs. RV group A.
than denopamine. In contrast, in 9 failing, Group B hearts the maximum response to isoproterenol was 1.7 times that of zinterol and 31 times that of denopamine. In the 9 failing, Group B hearts the response to zinterol was only marginally reduced by 34% (p is NS when compared to Group A), while the response to isoproterenol was reduced by 57.5% (p < .05). In Group B ventricles denopamine produced little or no response, with the mean response reduced by 98% compared to Group A hearts (p < .05).

Discussion

β-adrenergic receptors exist in multiple structural forms, as was first suggested by Lands et al. and Furchgott. In the Lands classification β-adrenergic receptors linked to cardiac stimulation and lipolysis were designated as "B-1," while receptors present in vascular and bronchial smooth muscle were designated "B-2." Lands' original classification has proven extremely useful, but it probably does not completely account for the structural complexity of β-adrenergic receptor subpopulations and it does not account for species differences in the assignment of organs to the β₁ or β₂ category.

In the current investigation computer modeling of selective and nonselective antagonist–radioligand competition curves in membranes derived from human left and right ventricular myocardium indicates that these tissues contain both β₁- and β₂-adrenergic receptors, in agreement with previous studies in human ventricular myocardium. The predominant receptor was the β₁ subtype, as indicated by the β₁-selective antagonist betaxolol binding with a nM-range Kᵢ value. The β₂ receptor was the minority receptor popula-
BVF subjects in this investigation were on multiple medications, none of them were taking agents with a direct effect on $\beta_2$-adrenergic receptors. Moreover, subjects with primary pulmonary hypertension and isolated right ventricular failure were on medications similar to the BVF group; these subjects had LV values indistinguishable from donor LV or RV, and RV values identical to BVF RV or LV. Consequently, the $\beta$ receptor abnormalities present in failing ventricular myocardium cannot be due to medications taken by heart failure patients or to other systemic factors present in heart failure. The fact that $\beta_2$ receptors, membrane markers and creatine kinase activity were not decreased in failing human left ventricle indicates that $\beta_2$-adrenergic receptor down-regulation is a specific pharmacologic process, rather than being a nonspecific phenomenon secondary to diseased tissue. Therefore, it is highly likely that the encountered differences in $\beta_2/\beta_1$ receptor distribution were due to heart failure rather than to other factors.

In a tissue with a heterogeneous cellular constituency it is important to prove that the receptor subtypes identified by radioligand binding are present on the cell type selected for study. This was accomplished by demonstrating that muscle contraction in isolated tissue taken from failing right ventricles could be stimulated through both $\beta_2$ and $\beta_1$ mechanisms. The effect of the nonselective $\beta$ agonist isoproterenol was antagonized by a selective $\beta_1$, blocking dose of betaxolol, while stimulation of muscle contraction by the selective $\beta_2$ agonist zinterol was not antagonized by betaxolol and was blocked by the selective $\beta_2$ antagonist ICI 118,551. The effect of zinterol, therefore, was confined to $\beta_2$ receptor stimulation. Quantitative aspects of the blockade of isoproterenol by the selective $\beta_2$ antagonist betaxolol suggested that the nonselective agonist isoproterenol produced stimulation of muscle contraction through both $\beta_1$ and $\beta_2$ receptors. Based on the discrepancy between $K_a$ values calculated from muscle contraction and $K_i$'s generated from competition curves, it appeared that the stimulation of muscle contraction by isoproterenol was not strictly $\beta_1$, as betaxolol produced a shift in the isoproterenol dose-response curve that was significantly less than that predicted from ICYP–betaxolol competition curves. The most likely explanation for this finding is that
isoproterenol was also stimulating muscle contraction through a $\beta_2$ mechanism that was not antagonized by the selective $\beta_1$ blocking dose of betaxolol.

The maximum inotropic effects of selective and nonselective $\beta$ agonists in isolated tissue were consistent with a selective down-regulation of $\beta_2$-adrenergic receptors in failing ventricular myocardium. Denopamine (TA064) is a selective $\beta_1$ agonist that does not produce a positive inotropic response in $\beta_2$-containing frog myocardium but produces a positive inotropic effect in $\beta_1$-containing rabbit myocardium that is antagonized by betaxolol to a degree predicted by $\beta_2$ stimulation (unpublished observations). Although the response to denopamine was 66% of the isoproterenol response in nonfailing ventricle, in failing heart denopamine produced little or no positive inotropic effect. These observations are consistent with denopamine's classification as a selective $\beta_1$ partial agonist$^{20,24}$ with loss of efficacy$^{20}$ in failing myocardium due to marked $\beta_2$ receptor down-regulation. In contrast, the response to zinterol was sustained in failing heart, as the 34% decrease in maximum inotropic effect was not statistically different from nonfailing preparations. As a result, the zinterol/isoproterenol maximum effect ratio increased from 0.39 in nonfailing ventricle to 0.60 in preparations derived from failing heart. The 34.3% decrease in the zinterol maximum in preparations derived from failing heart compares to the statistically insignificant, 18.9% reduction in $\beta_2$ receptor density in failing ventricular myocardium. The reduction in isoproterenol maximum in failing tissue was by 57.5%, compared to a reduction in total $\beta_2$ receptor density of 51.2% in membranes derived from failing ventricular chambers.

Thus in failing vs. nonfailing human ventricular myocardium there was good agreement between 1) the reduction in total number of radioligand-measured $\beta_2$-adrenergic receptors ($\beta_2$ plus $\beta_3$) and the isoproterenol maximum; 2) the small, statistically insignificant reductions in measured $\beta_2$, adrenergic receptors and zinterol maximal response, and 3) the marked reduction of measured $\beta_2$-adrenergic-receptor density and near total loss of activity of the $\beta_1$-partial-agonist denopamine. Collectively, these data make it likely that the measured values of $\beta_2$-adrenergic-receptor subpopulations in nonfailing and failing myocardium accurately reflect the concentrations of these receptors on myocardial cells.

Our data indicate that the $\beta_2$ subpopulation constitutes a substantial portion of the total number of $\beta_2$-adrenergic receptors present on failing human ventricular myocardial cells. In individuals with heart failure it would be expected that the myocardial $\beta_2$ receptor could be used for endogenous support of contractile function, particularly in response to an elevation in circulating epinephrine. The $\beta_2$ receptor population could also be used for pharmacologic support using nonselective $\beta$ or selective $\beta_2$ agonists. The presence of a significant concentration of $\beta_2$ receptors in the failing human heart may partially explain previously reported favorable results of $\beta_2$ agonist administration in heart failure, results that were attributed to afterload reduction or stimulation of cardiac $\beta_1$ receptors.$^{41-46}$ The high proportion of down-regulation resistant $\beta_2$ receptors in the failing human heart may also be viewed as a "back-up system" to safeguard against complete loss of the $\beta$-receptor population as heart failure progresses.

The causes of selective down-regulation of $\beta_2$ receptors in failing human heart has not been directly addressed by this study. Preliminary data from our laboratory (Bristow MR and Ginsburg R, unpublished data) indicate that down-regulated $\beta_2$-adrenergic receptors are truly "lost" and not simply internalized inasmuch as lightweight vesicles from failing human myocardial membranes have a $\beta_2$-adrenergic-receptor density that is proportional to the concentration in heavier fractions. Interconversion of $\beta_2$ and $\beta_3$ receptors would also seem unlikely, as the myocardial $\beta_3$:fraction is unchanged in heart failure, rather than being increased. Increased exposure to norepinephrine, an agonist that has a tenfold higher affinity for $\beta_3$ receptors,$^{49}$ is one potential explanation for selective $\beta_2$ receptor down-regulation. Failing human heart is exposed to a high concentration of norepinephrine$^{50}$ that is probably locally regulated,$^{49}$ and therefore norepinephrine exposure could be responsible for $\beta_2$ receptor down-regulation. Previous data in rats bearing norepinephrine-secreting pheochromocytomas have indicated selective $\beta_2$ down-regulation in this system,$^{50}$ perhaps for the same reason. One possible interpretation of these findings, then, is that selective myocardial $\beta_2$-receptor down-regulation is a marker of the degree of prior norepinephrine exposure. Other explanations for selective $\beta_2$ down-regulation include innervation of $\beta_3$ receptors and not $\beta_2$ receptors$^{50}$ or inherent resistance of myocardial $\beta_3$ receptors to subsensitivity phenomena.

In summary: Human ventricular myocardium contains both $\beta_1$ and $\beta_2$ receptors, both of which are coupled to contraction. In nonfailing myocardium the predominant $\beta_1$ population mediates the majority of tension response to nonselective agonists. In heart failure $\beta_2$ receptors selectively down-regulate, which leaves a population that is 38% $\beta_3$. The selective down-regulation of the $\beta_2$-receptor population markedly reduces the ability of selective $\beta_2$ partial agonists to mediate a positive inotropic response; selective $\beta_2$ agonists retain near-full inotropic activity mediated through a $\beta_3$ population that is not significantly decreased. The relatively high percentage of $\beta_2$ adrenergic receptors in failing human ventricular myocardium has implications for the therapeutic use of $\beta$ agonists and antagonists, and for the role played by the adrenergic nervous system in the natural history of heart failure.

Appendix: Computer Modeling Methodology for Radioligand — Cold Ligand Competition Curves

The first method consists of modeling the curves with the LIGAND program$^{52}$ run on an HP 9816 computer (Hewlett Packard, Palo Alto, Calif.). In this program...
the best fit is determined by a Runs test showing no serial correlation, plus the presence of a significant \( (p < .05) \) F test between the more complex and next simplest fit.

The second method for modeling competition curves was a two-step process involving an initial fit of the data to a 4-parameter logistic equation:\(^5\)

\[
B = B_{\text{max}} + (B_{\text{max}} - B_{\text{min}})/(1 + (X/K_{D1} + A))
\]

where \( B = \) bound radioligand, \( B_{\text{max}} = \) the bottom of the competition curve, or nonspecific binding, \( B_{\text{max}} = \) the top of the competition curve, \( X = \) cold ligand concentration, \( IC_{50} = ED_{50} \) of the curve, and \( h = \) Hill number or curve slope.

After derivation of these parameters binding data were converted to fraction of radioligand bound where \( 1 = 100\% \) of ICYP binding, or binding in the absence of displacing, cold ligand. The fractional binding data are then fitted to equations for 1, 2, and 3 site models; the equation for a 2-site fit is given below:

\[
\frac{\text{fraction bound}}{1 - a} = \frac{(X) (1 - A/(K_{D1} + A))}{K_{D1} (1 + (X)/(1 - a/(K_{D2} + A)))} - b
\]

where \( X = [\text{cold ligand}], A = [\text{radioligand}], K_{D1} = \text{radioligand dissociation constant, } K_{D2} = \text{dissociation constant for cold ligand and site 1, } a = \text{proportion of receptors that are of site 1, } K_{D2} = \text{dissociation constant for cold ligand and site 2, } b = \text{proportion of receptors that are of site 2.} \)

The total number of receptors and \( K_{D2} \) are determined from a saturation binding curve of specifically bound radioligand vs. increasing radioligand concentration, at the same time as the competition curve is performed. The value \( A/(K_{D1} + A) \) is determined from the saturation curve and independently entered as a fixed value. This method is valid only if \( A > > K_{D1} \), which is accomplished by keeping \( A \geq 5 \times K_{D2} \) (if \( A \) is not \( > > K_{D1} \), free \( A \) at the higher end of the competition curve will be \( > A \) at the low end). The criteria for the best fit are a \( p < .05 \) (t test) for each site and a higher \( F \) value than for other fits.

This second program runs on a DEC10 computer accessed by the Prophet system using a modern, terminal emulator, and an HP9816 computer.\(^9\) The modeling program used by Prophet is the MLAB program,\(^{12}\) and this method will subsequently be referred to as “MLAB.” The first phase of the MLAB analysis, modeling of dose-response curve data to a 4-parameter logistic equation, was also used to analyze dose-response data from muscle bath experiments.

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**β-Receptor Subpopulations in the Human Heart**


**Key Words** • β-adrenergic receptor subpopulations • human ventricular myocardium • radioligand binding
Beta 1- and beta 2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective beta 1-receptor down-regulation in heart failure.

M R Bristow, R Ginsburg, V Umans, M Fowler, W Minobe, R Rasmussen, P Zera, R Menlove, P Shah and S Jamieson

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