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 β₂ 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)

P REVIOUS studies using radioligand binding techniques have shown that human myocardial tissue contains a relatively high proportion of the β₁-adrenergic-receptor subtype. 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 myocardium and only small percentages of β₂ receptors have been identified in atrial tissue of some mammalian laboratory animal species. Limited tissue bath data in isolated human ventricular myocardium suggest that the β₂-adrenergic receptor is coupled to a positive inotropic response. Such information is essential for establishing a cardiomyo-cytic 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 β-adrenergic receptor population in human ventricular myocardium. Failing human left ventricle has a decreased total population of β receptors, which confers subsensitivity to β-receptor-mediated mechanical events. 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. In 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 nonse-
lective 0-agonist isoproterenol, the selective 0,-agon-
ist denopamine (TA604)20 and the selective 0,-agon-
ist zinterol.21 Agonists were administered in 10 cumula-
tive doses between 10-4 and 10-5 M. Additionally, in
20 BVF hearts the effects of the selective 0,-antago-
nist betaxolol22 and the selective 0,-antagonist ICI
118,55123 on the dose-response to isoproterenol or
zinterol was assessed in isolated RV trabeculae. An-
tagonsists 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.14,15 Response was re-
corded as the net increase in milligrams tension, de-
bined 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 homog-
emized with a Polytron (Brinkmann Instruments, West-
bury, 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 50,000g pellet, as previously de-
scribed. In 7 hearts (—) [123]iodocyanopindolol (ICYP)
binding isotherms (see below) were measured in
fleshly 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 0-receptor density as
judged from a comparison of 7 LV preparations as-
sayed immediately at the time of harvest to the same
preparations assayed later after storage. Relative to
the washed 50,000g pellet of the homogenate, left ventric-
ular membranes prepared in this manner had a
3.1 ± 0.6 fold enrichment in 0-adrenergic receptor
density (ICYP binding).

For receptor assays the total population of 0-adre-
ergic receptors was radiolabeled with ICYP using pre-
viously described methods.19 Assays were con-
ducted 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 (aver-
age 123 ± 12 g/ml). Maximum binding (Bmax) and
dissociation constant (Kd) were determined by nonlinear
least-squares fit of the specific binding curve, as
previously described.24 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-
tion into non-0-receptor active products in direct rela-
tion to radioactive decay.25

0, versus 0,-adrenergic receptor subtypes were
identified by computer modeling of 17-19 point beta-
ol-ICYP competition curves performed in each
preparation. Additionally, in 9 LV and 4 RV Group A
preparations 17-19 point ICI 118,551-ICYP competi-
tion curves were also performed, and in 12 LV pre-
parations (±) propranolol-ICYP competition curves
were performed. Assay conditions for competition
curves were identical to those for ICYP specific bind-
ing 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
(Kd values) of 0,-antagonists were derived from the
equation

\[
K_d = \frac{[\text{antagonist}]}{(\text{dose ratio} - 1)}
\]

Adenylate cyclase in crude membranes and homog-
enates was measured as previously described,14 while
5' nucleotidase activity was measured by the method
of Ipata.27 Creatine kinase activity in soluble fractions
of spun homogenates was determined as previously
described.14 Proteins were measured by the Peterson
modification of the Lowry technique.28

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,29 Khatri,30 and Grizzle
and Allen31 and summarized by Morrison.32 This anal-
ysis 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 decom-
position, 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 com-
puters, Boca Raton, Fla.). To detect potential differ-
ences in dose-response curves, this analysis was ap-
plied to points between the ED95 and ED50 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 test33 was employed.
Chemicals and Radioisotopes
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 VENTRICLES): LEFT VENTRICLE. In preparations derived from nonfailing left ventricle betaxolol, a selective $\beta_2$ competitive antagonist, yielded comparable values for proportion of $\beta_2$- 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 $\beta_2$- 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, $\beta_2$ 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 A (NONFAILING VENTRICLES): RIGHT 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 $\beta_2$/$\beta_2$ (MLAB), .765 ± .040: .232 ± .033; $\beta_2$/$\beta_2$ receptor density calculated from ICYP $B_{\text{max}}$, 61.7 ± 11.9: 17.2 ± 2.1 fmol/mg.

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

GROUP B (FAILING VENTRICLES): LEFT 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 $K_D$ ($K_D$) 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

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In 9 nonfailing left ventricles (5 donors and 4 from PPH subjects) ICYP–ICI 118,551 competition curves were also performed. A representative experiment is shown in Figure 2, and mean values are compared to betaxolol–ICYP competition curve data in Table 4. ICY

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>$B_{\text{max}}$ (fmol/mg)</th>
<th>$K_D$ (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, nonfailing</td>
<td>88.0</td>
<td>8.72</td>
</tr>
<tr>
<td>(12)</td>
<td>± 7.3</td>
<td>± 1.00</td>
</tr>
<tr>
<td>B, failing</td>
<td>43.2*</td>
<td>10.39†</td>
</tr>
<tr>
<td>(12)</td>
<td>± 3.41</td>
<td>± 1.22</td>
</tr>
</tbody>
</table>

*p < .0001 vs. respective subgroup in A. †p > .05 vs. respective subgroup in A.

Identification and Quantification of $\beta_1$ and $\beta_2$ Populations

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<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</td>
<td>10.5 ± 1.2</td>
<td>14.3 ± 2.9</td>
</tr>
<tr>
<td>(nmol/min/mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Adenylate cyclase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(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</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(IU/g wet wt.)</td>
<td>1063 ± 83</td>
<td>909 ± 70</td>
</tr>
</tbody>
</table>

None of the differences were statistically significant.
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 $\beta_2$ receptors is lower and the proportion of $\beta_1$ 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 respective relative proportions of $\beta_1$ and $\beta_2$ receptors; .664 and .337 for LIGAND (both $p<.001$ vs. nonfailing) 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 $\beta_2$-adrenergic receptors. On the other hand, $\beta_1$-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 LV: fraction $\beta_1$:$\beta_2$ (MLAB), .592 ± .063: .396 ± .060; $\beta_1$:$\beta_2$ receptor density calculated from ICYP $B_{max}$, 21.4 ± 1.4:14.8 ± 3.8. 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.

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

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Curve slope (Hill #)</th>
<th>MLAB Fraction</th>
<th>$K_i$ (nM)</th>
<th>Ligand Fraction</th>
<th>$K_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta_1$</td>
<td>$\beta_2$</td>
<td>$K_i(K_H)$</td>
<td>$K_i(K_L)$</td>
<td>$\beta_1$</td>
</tr>
<tr>
<td>A, nonfailing</td>
<td>0.74</td>
<td>0.773</td>
<td>0.228</td>
<td>5.01</td>
<td>210</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>
</tr>
<tr>
<td>B, failing</td>
<td>0.64*</td>
<td>0.600†</td>
<td>0.386†</td>
<td>5.25‡</td>
<td>235‡</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>
</tr>
</tbody>
</table>

* $p > 0.01$ vs. respective subgroup in A.
† $p < 0.0001$ vs. respective subgroup in A.
‡ $p > 0.10$ vs. respective subgroup in A.
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 receptor density by 51.2%, the average decrease in β, 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 β, 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 β2 properties, and in human ventricular trabecula 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 ED50 and ED90 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 Kᵦ value calculated for betaxolol antagonism of isoproterenol from the mean curves shown in Figure 4 was 5.16 × 10⁻⁴ M. The Kᵦ values for betaxolol antagonism of isoproterenol calculated from individual muscle bath experiments was significantly greater than the high affinity Kᵦ value calculated from betaxolol ICYP competition curves: (Kᵦ ± SEM = 4.29 ± 2.73 × 10⁻⁴ M, Kᵦ (MLAB) 5.10 ± 0.56 × 10⁻⁴; p < .05).

Figure 5 shows the effect of zinterol is markedly antagonized by ICI 118,551, which produced a > tenfold 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 × 10⁻⁴ M was reached it yielded a significantly different (p < .05) slope comparing points between the control curve ED50 and the ED90 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 concen-
trations of $10^{-4}$ and $3 \times 10^{-8}$ 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).

FIGURE 3. Competition of betaxolol or propranolol with ICYP in membranes derived from a failing left ventricle. Slope factors were: betaxolol 0.66, propranolol 0.91. Two-site fit parameters for betaxolol by MLAB program are given on figure: LIGAND parameters were: fraction $B_1$, 0.683, $B_2$ 0.316, $K_B$ 9.02 nM, $K_L$ 264 nM. Both MLAB and LIGAND yielded a one-site fit for the propranolol curve.

**Effect of Nonselective and Selective β 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, 23–34 the selective $\beta_2$-partial-agonist zinterol, 22, 35 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 the response to 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>Saturation curves</th>
<th>Betaxolol–ICYP competition curves</th>
<th>Subpopulation receptor density (fmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total β receptor density (fmol/mg)</td>
<td>Fraction $\beta_1$</td>
</tr>
<tr>
<td>Group</td>
<td>ICYP $B_{max}$</td>
<td>$B_1$</td>
</tr>
<tr>
<td>A. (Nonfailing)</td>
<td></td>
<td>$B_1$</td>
</tr>
<tr>
<td>1. LV</td>
<td>88.0</td>
<td>±7.3</td>
</tr>
<tr>
<td>n = 12</td>
<td></td>
<td>±0.028</td>
</tr>
<tr>
<td>2. RV</td>
<td>78.8</td>
<td>±13.0</td>
</tr>
<tr>
<td>n = 4</td>
<td></td>
<td>±0.776</td>
</tr>
<tr>
<td>3. LV + RV</td>
<td>85.7</td>
<td>±6.2</td>
</tr>
<tr>
<td>n = 16</td>
<td></td>
<td>±0.771</td>
</tr>
<tr>
<td>B. (Failing)</td>
<td></td>
<td>$B_1$</td>
</tr>
<tr>
<td>1. LV</td>
<td>43.2*</td>
<td>±3.4</td>
</tr>
<tr>
<td>n = 12</td>
<td></td>
<td>±0.024</td>
</tr>
<tr>
<td>2. RV</td>
<td>40.0†</td>
<td>±4.6</td>
</tr>
<tr>
<td>n = 9</td>
<td></td>
<td>±0.607†</td>
</tr>
<tr>
<td>3. LV + RV</td>
<td>41.8*</td>
<td>±2.7</td>
</tr>
<tr>
<td>n = 21</td>
<td></td>
<td>±0.603*</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 Kᵣ value. The β₂ receptor was the minority receptor popula-
tion in most hearts, and was identified by a nM-range 
K_i value for the selective β_2 antagonist ICI 118,551.
Betaxolol was able to identify a second, low-affinity
receptor that was consistent with the β_2 subtype, and
ICI 118,551 was able to identify a second low-affinity
receptor that was consistent with the β_1 subtype. Compar-
ison of relative values of β_1/β_2 receptor populations
using betaxolol and ICI 118,551 as competing ligands for
ICYP gave very comparable values, even though these
antagonists possess "mirror image" selectivity. Also, the comparison of two methods of computer
modeling yielded comparable values for proportions of
β_1 and β_2 receptors and K_i values. It is therefore un-
likely that any artifact related to generation or analysis
of receptor binding data could have accounted for the
detection of apparent β_1- and β_2-adrenergic receptor
subpopulations in human ventricular myocardium.
Collectively, the data indicate that human ventricular
myocardium contains a mixed population of β_1 and β_2
receptors.

Heart failure had a dramatic effect on the proportion
of β_1 vs. β_2 receptors. In normal ventricular chambers
the ratio of β_1:β_2 was 77:23, while in failing chambers it
was 60:38. This near two-fold increase in the propor-
tion of β_2 receptors was not due to an increase in
their total concentration, but rather was due to a “selective”
down-regulation of the β_2 receptor population.
This led to a total concentration of β_1 receptors in
failing ventricular chambers that averaged only 38.1% of
the value in nonfailing chambers (p < .001). On the
other hand, the overall concentration of β_2 receptors in
failing chambers was not significantly decreased, and
was 81.1% of the concentration in nonfailing cham-
bers (p = NS).

The two subject populations were of similar age,
and sex distribution did not affect results. Although the
BVF subjects in this investigation were on multiple
medications, none of them were taking agents with a
direct effect on β-adrenergic receptors. Moreover, sub-
jects 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 β
receptor abnormalities present in failing ventricular
myocardium cannot be due to medications taken by
heart failure patients or to other systemic factors pres-
ent in heart failure. The fact that β_1 receptors, mem-
brane markers and creatine kinase activity were not
decreased in failing human left ventricle indicates that
β_1-adrenergic receptor down-regulation is a specific
pharmacologic process, rather than being a nonspecif-
ic phenomenon secondary to diseased tissue. There-
fore, it is highly likely that the encountered differences in
β_1/β_2 receptor distribution were due to heart failure
rather than to other factors.

In a tissue with a heterogenous cellular constitu-
eny 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 tis-
ue taken from failing right ventricles could be stimu-
lated through both β_1 and β_2 mechanisms. The effect of
the nonselective β agonist isoproterenol was antago-
nized by a selective β_1, blocking dose of betaxolol,
while stimulation of muscle contraction by the selec-
tive β_1 agonist zinterol was not antagonized by betaxo-
lol and was blocked by the selective β_2 antagonist ICI
118,551. The effect of zinterol, therefore, was con-
fined to β_2 receptor stimulation. Quantitative aspects of
the blockade of isoproterenol by the selective β_1
agonist betaxolol suggested that the nonselective
agonist isoproterenol produced stimulation of muscle
contraction through both β_1 and β_2 receptors. Based on
the discrepancy between K_i values calculated from
muscle contraction and K_i's generated from competi-
tion curves, it appeared that the stimulation of muscle
contraction by isoproterenol was not strictly β_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

![Figure 6. Comparison of the maximum net mg tension response of denopamine, zinterol and isoproterenol in right ventricle taken from seven nonfailing, normally functioning (NL) donor hearts and nine hearts with severe biventricular failure (HF). Specific values are (mean ± SEM); denopamine, NL 1510 ± 810, HF 17 ± 17 (p < .05); zinterol NL 886 ± 175, HF 582 ± 128 (p = NS); isoproterenol, NL 2280 ± 401, HF 969 ± 110 (p < .01).](image-url)
isoproterenol was also stimulating muscle contraction through a β₁ mechanism that was not antagonized by the selective β₂ blocking dose of betaxolol.

The maximum inotropic effects of selective and nonselective β agonists in isolated tissue were consistent with a selective down-regulation of β₂-adrenergic receptors in failing ventricular myocardium. Denopamine (TA064) is a selective β₁ agonist that does not produce a positive inotropic response in β₂ containing frog myocardium but produces a positive inotropic effect in β₁ containing rabbit myocardium that is antagonized by betaxolol to a degree predicted by β₂ 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 β₁ partial agonist with loss of efficacy in failing myocardium due to marked β₁ receptor down-regulation. In contrast, the response to zintrol was sustained in failing heart, as the 34% decrease in maximum inotropic effect was not statistically different from nonfailing preparations. As a result, the zintrol/isoproterenol maximum effect ratio increased from .39 in nonfailing ventricle to .60 in preparations derived from failing heart. The 34.3% decrease in the zintrol maximum in preparations derived from failing heart compares to the statistically insignificant, 18.9% reduction in β₁ receptor density in failing ventricular myocardium. The reduction in isoproterenol maximum in failing tissue was by 57.5%, compared to a reduction in total β₁ 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 β₂-adrenergic receptors (β₁ plus β₂) and the isoproterenol maximum; 2) the small, statistically insignificant reductions in measured β₂ adrenergic receptors and zintrol maximal response, and 3) the marked reduction of measured β₂-adrenergic-receptor density and near total loss of activity of the β₁-partial-agonist denopamine. Collectively, these data make it likely that the measured values of β₂-adrenergic-receptor subpopulations in nonfailing and failing myocardium accurately reflect the concentrations of these receptors on myocardial cells.

Our data indicate that the β₂ subpopulation constitutes a substantial portion of the total number of β₂-adrenergic receptors present on failing human ventricular myocardial cells. In individuals with heart failure it would be expected that the myocardial β₂ receptor could be used for endogenous support of contractile function, particularly in response to an elevation in circulating epinephrine. The β₂ receptor population could also be used for pharmacologic support using nonselective β or selective β₂ agonists. The presence of a significant concentration of β₂ receptors in the failing human heart may partially explain previously reported favorable results of β₂ agonist administration in heart failure, results that were attributed to afterload reduction or stimulation of cardiac β₁ receptors. The high proportion of down-regulation resistant β₂ receptors in the failing human heart may also be viewed as a “back-up system” to safeguard against complete loss of the β-receptor population as heart failure progresses.

The causes of selective down-regulation of β₂ 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 β₂-adrenergic receptors are truly “lost” and not simply internalized insasmuch as lightweight vesicles from failing human myocardial membranes have a β₂-adrenergic-receptor density that is proportional to the concentration in heavier fractions. Interconversion of β₁ and β₂ receptors would also seem unlikely, as the myocardial β₁ fraction is unchanged in heart failure, rather than being increased. Increased exposure to norepinephrine, an agonist that has a tenfold higher affinity for β₂ receptors, is one potential explanation for selective β₂ receptor down-regulation. Failing human heart is exposed to a high concentration of norepinephrine that is probably locally regulated, and therefore norepinephrine exposure could be responsible for β₂ receptor down-regulation. Previous data in rats bearing norepinephrine-secreting pheochromocytomas have indicated selective β₂ down-regulation in this system, perhaps for the same reason. One possible interpretation of these findings, then, is that selective myocardial β₂-receptor down-regulation is a marker of the degree of prior norepinephrine exposure. Other explanations for selective β₂ down-regulation include innervation of β₁ receptors and not β₂ receptors or inherent resistance of myocardial β₂ receptors to subsensitivity phenomena.

In summary: Human ventricular myocardium contains both β₁ and β₂ receptors, both of which are coupled to contraction. In nonfailing myocardium the predominant β₁ population mediates the majority of tension response to nonselective agonists. In heart failure β₁ receptors selectively down-regulate, which leaves a population that is 38% β₂. The selective down-regulation of the β₁-receptor population markedly reduces the ability of selective β₂, partial agonists to mediate a positive inotropic response; selective β₂ agonists remain near-full inotropic activity mediated through a β₂ population that is not significantly decreased. The relatively high percentage of β₂ adrenergic receptors in failing human ventricular myocardium has implications for the therapeutic use of β 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 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 < 0.05) 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

\[ B = \frac{B_{\text{max}} + (B_{\text{max}} - B_{\text{min}})(1 + (X/K_{C50}))^3}{1 + (X/K_{C50})^3} \]

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, \( K_{C50} \) = 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:

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

where \( X \) = [cold ligand], \( A \) = [radioligand], \( K_{D1} \) = radioligand dissociation constant, \( K_{H2} \) = dissociation constant for cold ligand and site 1, \( a \) = proportion of receptors that are of site 1, \( K_{D2} \) = dissociation constant for cold ligand and site 2, \( b \) = proportion of receptors that are of site 2.

The total number of receptors and \( K_{D} \) 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 \( A/\left( K_{D} + A\right) \) is determined from the saturation curve and independently entered as a fixed value. This method is valid only if \( A >> K_{D} \), which is accomplished by keeping \( A \approx 5 \times K_{D} \) (if \( A \) is not >> \( K_{D} \), 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 < 0.05 (r 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 modem, terminal emulator, and an HP9816 computer. The modeling program used by Prophet is the MLAB program, 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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