Delineation of the Distribution of 
\(\beta\)-Adrenergic Receptor Subtypes in 
Canine Myocardium

Sidney S. Murphree and Jeffrey E. Saffitz

\(\beta\)-Receptors constitute only 10–30% of the total \(\beta\)-adrenergic receptors in mammalian ventricular myocardium, but their precise tissue location cannot be determined easily by measuring physiological variables. To delineate the distribution of \(\beta\)-receptor subtypes in myocytic and vascular components of the heart, we incubated transmural sections of canine left ventricle with \([125I]\text{cyanopindolol}\) and selected concentrations of the \(\beta_1\)-selective antagonist betaxolol or the \(\beta_2\)-selective antagonist ICI 118,551. Detailed competition binding data were best accounted for by a two-site model in which approximately 75% of total sites were \(\beta_1\)- and 25% were \(\beta_2\)-receptors. The relative proportions of \(\beta\)-receptor subtypes in myocytic and vascular components were assessed autoradiographically by analyzing the density of binding sites in transmural sections incubated with radioligand and subtype-selective displacers. Betaxolol (10^{-7} M) reduced the density of radioligand binding sites by 44% in regions composed primarily of ventricular myocytes but by <5% in small coronary arterioles. ICI 118,551 (10^{-7} M) reduced radioligand binding-site density by 18% in myocytic regions and by 55% in small arterioles. In myocytic regions, these data indicated a subtype composition of approximately 85% \(\beta_1\)- and 15% \(\beta_2\)-sites. In contrast, arterioles contained almost exclusively the \(\beta_2\)-subtype. The diameters of coronary vessels in which \(\beta_1\)-receptors were found to be selectively increased fell within a narrow range (mean ± SD, 35 ± 11 \(\mu\)m; range, 16–55 \(\mu\)m). Small mural arteries and venules did not contain a significantly higher proportion of \(\beta_2\)-receptors than adjacent myocytic regions. (Circulation Research 1988;63:117–125)

Materials and Methods

Materials

\((-\)\text{[125I]}\text{Cyanopindolol (ICYP) \(2,200 \text{Ci/mmol}\)}} was purchased from New England Nuclear, Boston, Massachusetts. The nonselective coronary arteries and the microvasculature. The coronary arteries are of particular importance because of their role in regulation of vascular resistance and their potential role in the pathophysiology of heart failure.

To delineate the distribution of \(\beta\)-adrenergic receptors in specific tissue components of the heart, we have recently developed methods for quantitative, autoradiographic localization of radioligands bound to receptors in transmural slices of myocardium. In the present studies, we have used the \(\beta\)-adrenergic radioligand \([125I]\text{cyanopindolol}\) and the subtype-selective displacers betaxolol and ICI 118,551 to characterize the distribution of \(\beta\)-adrenergic receptor subtypes in transmural slices of canine myocardium. We identified a specific class of myocordial vessels, the small arterioles 20–70 \(\mu\)m in diameter, that contains almost exclusively the \(\beta_2\)-subtype.

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

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

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displacer l-propranolol HCl was a gift from Wyeth-Ayerst Laboratories, New York, New York; the β1-selective drug betaxolol was kindly provided by Synthelabo, Paris, France; the β2-selective antagonist ICI 118,551 HCl was a gift from Imperial Chemical Industries, Cheshire, England. All other reagents used were of the highest available purity.

Preparation of Tissue Sections

Hearts were excised rapidly from adult mongrel dogs anesthetized with thiopental (10 mg/kg i.v.) and rinsed briefly in saline at 4° C. Transmural blocks of the left ventricular free wall were dissected, cooled on dry ice, submerged slowly in liquid nitrogen and stored in sealed containers at -70° C until used in experiments. Frozen sections 12 μm in thickness were prepared from unfixed transmural blocks of tissue and mounted on gelatin-coated slides. Three serial sections were placed on each slide.

Radioligand Binding Assays

Unfixed slide-mounted transmural sections of myocardium were incubated for 120 minutes at 37° C in buffer (mM: NaCl 154, MgCl 10, Tris-HCl 10, pH 7.4) containing 8 pM ICYP and 20 separate concentrations (10^{-7} to 10^{-11} M) of the unlabeled displacers betaxolol or ICI 118,551. Betaxolol competition curves were analyzed in four separate experiments with tissue from four separate animals. ICI 118,551 competition curves were determined in three experiments with tissue from three animals.

A uniform radioligand concentration of 8 pM (approximately half Kd) was chosen to ensure a high specific-binding ratio (>90%) and facilitate comparisons of autoradiographic grain densities. Nonspecific binding was defined as binding of radioligand in the presence of 10^{-5} M l-propranolol. Binding assays were carried out in large volumes (40-50 ml) so that the concentration of free radioligand did not change measurably during the binding reactions. Nonspecifically bound radioactivity was removed by incubating slide-mounted tissue sections in buffer not containing radioligand or unlabeled displacer for 60 minutes at 37° C. The sections were then dipped briefly in distilled water to remove buffer solutes, dried under a gentle stream of air, and either scraped off slides for quantification of radioactivity with gamma scintillation spectrometry or prepared for autoradiography as described below. In previous studies, we have demonstrated that these binding procedures lead to equilibrium conditions and that rinsing results in selective removal of nonspecifically bound radioactivity without measurable removal of receptor-bound radioactivity.

Total radioactivity per section was determined by scraping sections from the slides with a razor and by quantifying radioactivity in each with gamma scintillation spectrometry (66% counting efficiency). In each individual experiment, radioactivity measurements were normalized to account for modest variations in section area that occurred during preparation of large numbers of sections of irregularly shaped tissue blocks. Total cross-sectional area and tissue protein content were measured in groups of sections selected at regular intervals during the preparation of serial sections in each individual experiment. Cross-sectional areas were measured by digitizing the outlines of enlarged photographs of the sections. Total tissue protein content was measured in individual sections scraped from acid-washed (nongelatin-coated) slides with the Lowry assay with bovine serum albumin standards.

In all experiments, individual data points were calculated as means of triplicate determinations.

Quantitative Autoradiography

The distribution of radioligand binding sites was analyzed in tissue from three additional animals incubated with ICYP and either propranolol or betaxolol and in tissue from two additional animals incubated with radioligand and either propranolol or ICI 118,551. Binding sites were localized autoradiographically in transmural sections with the emulsion-coated coverslip method. Acid-washed, gelatin-coated coverslips were coated with Kodak NTB2 nuclear track emulsion (Eastman Kodak, Rochester, New York) and dried at room temperature for at least 3 hours. The emulsion-coated coverslips were glued at one end to slides containing radiolabeled sections. After exposure of the emulsion for approximately 96 hours, the unglued edge of each coverslip was gently lifted from the slides and the emulsion was developed with Kodak D19 developer (diluted 1:1 with water) for 4 minutes and fixed with Kodak fixer for 4 minutes at 25° C. After photographic processing, the tissue sections were stained with hematoxylin and eosin, and the coverslips were sealed permanently to the slides. The tissue and overlying developed grains in the emulsion layer were examined by light microscopy, photographed, and grain densities determined by counting grains per unit area of selected regions of the section.

Data Analysis

All data are expressed as mean ± SD unless indicated otherwise. The statistical significance of differences in grain density measurements in light microscopic autoradiographs was determined with analysis of variance with the SAS general linear models procedures. Competition binding curves generated by incubating transmural sections under equilibrium conditions with a constant concentration of radioligand and varying concentrations of subtype-selective displacers were analyzed with the iterative curve-fitting program (LIGAND) of Munson and Rodbard as modified for microcomputers by McPherson. With this method, initial estimates of selected binding constants in the defined model are iteratively refined by nonlinear least-squares curve-fitting techniques based on the Marquardt-
Levenberg modification of the Gauss-Newton method.\textsuperscript{23} When the weighted sum of the squares was minimized, final parameter estimates were generated and fitted to the actual data.

The extent to which betaxolol or ICI 118,551 inhibited binding of ICYP to $\beta_1$- and $\beta_2$-receptors was determined with the following equation of Neve et al.\textsuperscript{27} describing the inhibition of binding of a selective radioligand by a selective competing ligand:

$$B = \frac{B_{\text{max}1} \cdot L}{L + K_{d1} \cdot (1 + \frac{i}{K_{d1}})} + \frac{B_{\text{max}2} \cdot L}{L + K_{d2} \cdot (1 + \frac{i}{K_{d2}})}$$

where $B$ is the amount of radioligand bound; $B_{\text{max}1}$ and $B_{\text{max}2}$ are the densities of $\beta_1$- and $\beta_2$-receptors; $L$ is the concentration of radioligand; $i$ is the concentration of unlabeled displacer; $K_{d1}$ and $K_{d2}$ are dissociation constants of $\beta_1$- and $\beta_2$-receptors, respectively, for the radioligand; and $K_{d1}$ and $K_{d2}$ are dissociation constants of the receptors for the unlabeled competing ligands.

Although ICYP has been regarded generally as nonsubtype selective, the results of studies by Neve et al.\textsuperscript{27} have demonstrated that this ligand is approximately twofold selective for $\beta_2$-receptors. In our previous studies of $\beta$-receptor autoradiography with ICYP in canine myocardium,\textsuperscript{21} we measured a $K_d$ of 21.3 ± 1.6 pM by Scatchard analysis of binding isotherms. Because subtype selectivity is unlikely to be detected by analysis of saturation data unless the two classes of binding sites differ in affinity for radioligand by at least fivefold to sevenfold\textsuperscript{28} and because $\beta_2$-receptors make up approximately 75% of myocardial receptors, it is reasonable to designate the observed $K_d$ of 21.3 pM as that of $\beta_2$-receptors for ICYP ($K_{d2}$ in the above equation).

Accordingly, $K_{d1}$ is equal to 10.7 pM (half of $K_{d2}$). Values for $K_{d1}$ and $K_{d2}$ were determined by analysis of detailed competition binding curves as described above and are shown in Table 1. With these values and values for the concentrations of radioligand ($L$), of displacing ligand ($i$), and of specific binding ($B$), the relative inhibition of ICYP binding to $\beta_1$- and $\beta_2$-receptors was calculated.

The proportions of $\beta_1$- and $\beta_2$-receptors in myocytic regions and vascular components were determined autoradiographically by comparing grain densities of corresponding binding sites in sections incubated with ICYP ± $l$-propranolol (total $\beta_1$- and $\beta_2$-receptors) and those incubated with ICYP and a specific concentration of a subtype-selective displacer. Analysis of the extent to which ICYP binding was inhibited to $\beta_1$- and $\beta_2$-receptors by the displacer (determined with the equation above) and the extent to which binding-site densities were reduced by the displacer in each compartment permitted calculation of the subtype composition of the compartment.

**Results**

The ability of the $\beta_1$-selective antagonist betaxolol to inhibit specific binding of ICYP was assessed by incubating serial transmural sections of canine left ventricle with 8 pM ICYP and 10$^{-3}$ to 10$^{-11}$ M betaxolol. Analysis of the competition binding curve shown in Figure 1 with a nonlinear curve-fitting algorithm indicated that the data could be best accounted for by a two-site model in which 76% of the binding sites were $\beta_1$- and 24% were $\beta_2$-receptors (Table 1). Betaxolol bound to $\beta_1$-receptors with a 148-fold greater affinity than to $\beta_2$-receptors.

A similar experiment was performed with the $\beta_2$-selective displacer ICI 118,551. As shown in Figure 2 and Table 1, the data were best accounted for by a two-site model. The proportion of $\beta_1$- and $\beta_2$-receptors determined by analysis of the ICI 118,551 competition curve is in accordance with that obtained in betaxolol competition binding studies.

The relative proportions of $\beta_1$- and $\beta_2$-receptors in regions of the ventricular sections composed primarily of myocytes and in the coronary vasculature were determined autoradiographically. Grain densities were compared in sections incubated with ICYP with or without $l$-propranolol and those incubated with ICYP ± betaxolol. Points are mean±SD of four separate experiments. ICYP, [125$I$]iodofycyanopindolol.
FIGURE 2. Graph of inhibition of ICYP binding by ICI 118,551. In each experiment, triplicate transmural sections were incubated with 8 pM ICYP and selected concentrations of ICI 118,551 as indicated in text. Percentage of total specific binding was determined by comparing radioligand binding in presence of ICI 118,551 with difference in binding in presence and absence of $10^{-5}$ M propranolol. Points are mean±SD of three separate experiments. ICYP, [$^{125}$Iodo]cyanopindolol.

with ICYP and specific concentrations of subtype-selective blockers designed to maximally inhibit binding of radioligand to one subtype while minimally inhibiting binding to the other.

In initial experiments, $10^{-7}$ M betaxolol was selected. As this concentration, betaxolol inhibited binding of ICYP to $\beta_1$-sites by approximately 56%, whereas binding to $\beta_2$-sites was inhibited by approximately 1%. These values were determined with the equation described above. At a radioligand concentration of 8 pM in the absence of a selective competing ligand ($L = 8$ pM, $i = 0$), $B = 0.273 B_{\text{max}} + 0.428 B_{\text{max}}$. In the presence of $10^{-7}$ M betaxolol, $B = 0.119 B_{\text{max}} + 0.425 B_{\text{max}}$, indicating a reduction in binding of radioligand to $\beta_1$-sites of 56% (0.119 versus 0.273) but of only approximately 1% to $\beta_2$-receptors (0.425 versus 0.428).

Upon microscopic examination of the betaxolol-treated autoradiographs, it was readily apparent that certain blood vessels had an increased grain density in comparison with surrounding myocytic

FIGURE 3. Autoradiographs of transmural section of canine left ventricle prepared after incubation with 8 pM [$^{125}$Iodo]cyanopindolol and $10^{-7}$ M betaxolol as indicated in text. Panel A, low-power view, focused on the plane of tissue, including cross sections of small arterioles (b,c), a small intramural artery (d), and adjacent myocytic regions. Panels B, C and D, higher power views, focused on the plane of overlying emulsion, of vessels identified in Panel A with corresponding lower case letters. Small arterioles (approximate diameters 30–40 $\mu$m) have considerably higher overlying grain densities than surrounding myocytes. Artery (diameter approximately 300 $\mu$m) has a lower grain density than adjacent myocytes.
regions (Figures 3 and 4). To characterize the specific types of vessels exhibiting an increased grain density when binding of ICYP was inhibited selectively at $\beta_1$-sites, the morphology was noted, and the diameter was measured of each vessel having a grain density markedly greater than that of surrounding structures. The increased grain density was located exclusively overlying small coronary arterioles with diameters of 16–55 $\mu$m (mean diameter = 35 ± 11 $\mu$m, $n = 96$). Small intramural arteries (100–300 $\mu$m in diameter) and venules showed no apparent increase in grain density in comparison with adjacent myocytes.

The distribution of $\beta$-receptor subtypes in myocytic regions and small coronary arterioles was quantified by measuring relative grain densities. To facilitate comparisons, the grain density of total $\beta$-receptors in the myocytic component of the heart, measured in sections incubated with ICYP with or without propranolol, was normalized to 1 and all other grain densities were compared to this value (Table 2). Actual grain densities of total $\beta$-receptors in myocytic regions averaged 563 ± 83 grains/10$^4$ $\mu$m$^2$. Small coronary arterioles had a total $\beta$-receptor grain density approximately 1.42 times that observed in the ventricular myocytes. The grain density overlying ventricular myocytes was reduced by approximately 44% in sections incubated with $\beta$-blockers.

**Table 2. Relative Grain Densities of Myocytic Regions and Small Arterioles**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Myocytes</th>
<th>Arterioles</th>
<th>Myocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total $\beta$-receptors</td>
<td>1 ± 0.16</td>
<td>1.42 ± 0.13</td>
<td>1.42:1</td>
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<tr>
<td>(ICYP + 10$^{-5}$ M $\beta$-propranolol)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selective inhibition of $\beta_1$-sites (ICYP + 10$^{-7}$ M betaxolol)</td>
<td>0.56 ± 0.13</td>
<td>1.38 ± 0.17†</td>
<td>2.46:1</td>
</tr>
<tr>
<td>Total $\beta$-receptors</td>
<td>1 ± 0.16</td>
<td>1.48 ± 0.23</td>
<td>1.48:1</td>
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<tr>
<td>(ICYP + 10$^{-3}$ M $\beta$-propranolol)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selective inhibition of $\beta_1$-sites (ICYP + 10$^{-7}$ M ICI 118,551)</td>
<td>0.82 ± 0.14</td>
<td>0.67 ± 0.11</td>
<td>0.82:1</td>
</tr>
</tbody>
</table>

All values are mean ± SD. Grain densities of total and betaxolol-inhibited binding were measured in four tissue sections from two transmural blocks of left ventricle from three animals. Three to five individual measurements were made for each compartment in each section. Values of total and ICI 118,551-inhibited binding were obtained in seven sections from two blocks from two animals. Five to seven grain densities were measured for each compartment in each section. Total and displacer-inhibited binding values were adjusted by subtracting the grain densities of myocytes and arterioles due to nonspecific binding plus autoradiographic background, measured in sections incubated with ICYP + 10$^{-5}$ M $\beta$-propranolol.

*p<0.01 vs. arterioles.

**Figure 4. Autoradiographs of small arterioles and adjacent myocytes.** Upper panels focused on the plane of the section; lower panels, focused on the overlying emulsion. Tissue shown on left was incubated with 8 pM [125I]iodocyanopindolol (ICYP) only (specific binding >90%), and tissue on right was incubated with 8 pM ICYP and 10$^{-7}$ M betaxolol. Betaxolol caused a marked reduction in grain density overlying myocytic regions but little apparent change in grain density of the arterioles.
bated with 8 pM ICYP and 10⁻⁷ M betaxolol. In contrast, the grain density overlying the small coronary arterioles was reduced by less than 3%. Thus, the ratio of binding sites in small arterioles to myocytes was increased to approximately 2.46 when binding of ICYP to β₁-receptors was inhibited selectively by betaxolol.

These autoradiographic data were analyzed with the equation of Neve et al.²⁷ to calculate the proportions of β-receptor subtypes in myocytic regions and coronary arterioles (Table 3). In myocytic regions, the total specific grain density was equal to the sum of β₁- and β₂-receptors (1 = 0.273 • Bmax₁ + 0.428 • Bmax₂). In the presence of 10⁻⁷ M betaxolol, binding of ICYP in myocytic regions was reduced by 44% (grain density = 0.56) as the result of a 56% inhibition of binding to β₁-sites and 1% inhibition to β₂-sites (0.56 = 0.119 • Bmax₁ + 0.425 • Bmax₂). Solving these two equations simultaneously resulted in a calculated myocytic region subtype composition of 85% β₁ and 15% β₂-receptors. In small coronary arterioles, total specific grain density was 1.42 (1.42 = 0.273 • Bmax₁ + 0.428 • Bmax₂), whereas in the presence of 10⁻⁷ M betaxolol, the grain density was reduced to 1.38 (1.38 = 0.119 • Bmax₁ + 0.425 • Bmax₂). Solving the equations indicated that the subtype composition of arterioles was approximately 93% β₁ and 7% β₂.

These findings were confirmed in additional experiments in which grain densities were measured in sections incubated with 8 pM ICYP and 10⁻⁷ M ICI 118,551. At 10⁻⁷ M, ICI 118,551 inhibited binding of radioligand to β₂-sites by approximately 60% and to β₁-sites by approximately 5% (B = 0.260 • Bmax₁ + 0.168 • Bmax₂). As shown in Table 2 and Figure 5, the grain density overlying myocytic regions was reduced by 18% (0.82 versus 1), and the grain density of arterioles was reduced by 55% (0.67 versus 1.48) in sections incubated with 8 pM ICYP and 10⁻⁷ M ICI 118,551. Based on these observations, the subtype composition of myocytic regions was calculated to be approximately 84% β₁ and 16% β₂, while the subtype composition of small arterioles was calculated to be approximately 89% β₂ and 11% β₁ (Table 3).

The grain densities of total β-receptors overlying the large epicardial coronary arteries were approximately threefold to fivefold lower than those observed in regions composed primarily of ventricular myocytes (Figure 6). Thus, reliable estimates

<table>
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<th>Compartment</th>
<th>β₁ (%)</th>
<th>β₂ (%)</th>
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<tbody>
<tr>
<td>Betaxolol</td>
<td>Myocytic regions</td>
<td>85</td>
<td>15</td>
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<tr>
<td></td>
<td>Arterioles</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>ICI 118,551</td>
<td>Myocytic regions</td>
<td>84</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Arterioles</td>
<td>11</td>
<td>89</td>
</tr>
</tbody>
</table>

**Figure 5.** Representative autoradiographs of a transmural section of canine left ventricle prepared after incubation with 8 pM [¹²⁵I]iodo]cyanopindolol and 10⁻⁷ M ICI 118,551 as indicated in text. Upper panels, focused on tissue; lower panels, focused on emulsion.
of the subtype composition of the large coronary arteries could not be obtained in the autoradiographic preparations analyzed in the present study.

Discussion

We have used subtype-selective displacers and computer analysis of detailed competition binding curves to quantitatively characterize the distribution of $\beta_1$- and $\beta_2$-receptors in canine left ventricle. The design of the selective displacer competition studies was similar to that of more conventional studies with the exception that transmural tissue sections rather than aliquots of membranes were used. The results obtained in tissue sections regarding the relative proportion of $\beta$-subtypes in the left ventricle and the relative affinities of betaxolol and ICI 118,551 for $\beta_1$- and $\beta_2$-receptors are close to those reported in assays of membranes prepared from homogenates of whole myocardium.16,29-31 With the use of tissue sections, however, complex anatomic relations are maintained, and the distribution of $\beta$-receptor subtypes may be characterized autoradiographically with light microscopic resolution.

The results of this study indicate that a specific class of coronary vessels, coronary arterioles 20–70 $\mu$m in diameter, contains almost exclusively $\beta_2$-adrenergic receptors. Similar results have been reported in studies of isolated cerebral microvessels.32 Because the grain density of total binding sites over the large intramural and epicardial coronary arteries was significantly lower than over small arterioles (Figures 3 and 6, Murphree and Saffitz,21 and Muntz et al33), the $\beta$-receptor subtype composition of the large arteries could not be quantified reliably in the present experiments. Results of radioligand binding studies in membranes prepared from isolated coronary arteries indicate, however, that large arteries contain predominantly the $\beta_1$-subtype.16,17 Thus, the coronary conductance and resistance vasculature appear to have marked differences in $\beta$-receptor subtype composition.

Analysis of grain densities after selective inhibition of binding to $\beta_1$-sites with betaxolol indicated that while $\beta_2$-receptors constitute only approximately 25% of total canine myocardial $\beta$-receptors, they represent approximately 90% of receptors located in a particular class of small arterioles. These observations were confirmed in studies in which binding to $\beta_2$-sites was inhibited selectively with ICI 118,551. Although small coronary arterioles contain almost exclusively the $\beta_2$-subtype, these vessels comprise only a few percent of total cross-sectional area. Thus, a considerable proportion of myocardial $\beta_2$-receptors must be located in other compartments. Autoradiographic analysis indicated that myocytic regions have a subtype composition of approximately 85% $\beta_1$-receptors and 15% $\beta_2$-receptors. Because these regions make up >90% of the total sectional area, they must therefore contain a significant proportion of the $\beta_2$-receptors in the heart. These regions contain not only myocytes, but capillary endothelial cells and interstitial fibroblasts as well. The light microscopic methods that were used cannot resolve binding sites in individual closely apposed cells. Thus, our results do not prove that both $\beta_1$- and $\beta_2$-receptors are located on individual ventricular myocytes. Although some $\beta_2$-receptors localized to myocytic regions may be located on fibroblasts34 or endothelial cells,35 recent studies in human myocardium suggest that $\beta_2$-receptors mediate a positive inotropic response.31,36,37

Our observations extend the original autoradiographic studies of Muntz et al33 who showed that selected concentrations of the $\beta_1$-antagonist meto-
prol, inhibited binding of $[^3H] $dihydralpranolol to cardiac myocytes to a greater extent than to arterioles. Their results indicated that the proportion of $\beta_1$-receptors in myocytes was considerably higher than that in arterioles. The present study extends these observations by the use of quantitative methods to analyze competition curves and autoradiographic preparations designed to delineate the proportions of $\beta_1$- and $\beta_2$-receptors in specific components of the ventricle.

Recently, Chilian et al.\(^{18}\) have demonstrated that both large and small coronary vessels contribute to resting coronary vascular resistance. Approximately 25% of total resistance is attributed to vessels larger than 200 $\mu$m in diameter and 45% to vessels larger than 100 $\mu$m. Our demonstration of a markedly different $\beta$-receptor subtype composition in coronary vessels 16–55 $\mu$m in diameter versus larger vessels suggests that disparate mechanisms may potentially regulate coronary resistance and blood flow in these components of the vasculature.

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