Autoradiographic Localization of \( \beta \)-Adrenergic Receptors in Human Large Coronary Arteries

F. Amenta, L. Coppola, P. Gallo, F. Ferrante, A. Forlani, A. Monopoli, and P. Napoleone

The distribution of \( \beta \)-adrenergic receptors in sections of the human right and left coronary arteries and of the anterior intraventricular branch was studied by the use of combined in vitro radioreceptor binding and autoradiographic techniques. \( ^{125}\)I]Cyanopindolol was used as a ligand for \( \beta \)-adrenergic receptors. Binding of the radioligand to sections of the three coronary arteries under study was saturable, stereoselective, reversible, and displaceable by antagonists and agonists with the rank order of potency expected for \( \beta \)-adrenergic receptors. Analysis of binding isotherms indicated maximum binding capacities of 41.5 fmol/mg protein for the right coronary artery, 35.4 fmol/mg protein for the left coronary artery, and 25.7 fmol/mg protein for the anterior intraventricular branch. Dissociation constants were -35 pM in the arteries examined. The relative amounts of \( \beta_1 \)- and \( \beta_2 \)-receptor subtypes were as follows: 72% \( \beta_1 \)-receptors and 28% \( \beta_2 \)-receptors in the right coronary artery; 65% \( \beta_1 \)-receptors and 35% \( \beta_2 \)-receptors in the left coronary artery; 40% \( \beta_1 \)-receptors and 60% \( \beta_2 \)-receptors in the anterior intraventricular branch. The results of autoradiographic analysis revealed a predominance of \( \beta \)-receptors in the medial layer. \( \beta \)-Receptors were localized primarily in the adventitia, in the adventitia-media border, and in the intimal layer. These results should lead to a better understanding of the mechanisms involved in the control of coronary circulation in humans. (Circulation Research 1991;68:1591–1599)

The role of \( \beta \)-adrenergic receptors in the control of large coronary vasomotion is debated (for a review see Reference 1). In vitro studies demonstrated that activation of \( \beta \)-adrenergic receptors results in coronary artery relaxation.\(^2\)\(^-\)\(^4\) However, it is not certain whether the same is true in vivo. In fact, the analysis of the effects of \( \beta \)-adrenergic receptor stimulation on coronary resistance vessels is made difficult by the existence of a vasodilation secondary to the increase in myocardial metabolic demands elicited by \( \beta \)-adrenergic receptor agonists.\(^2\)\(^-\)\(^4\) Moreover, large epicardial coronary arteries are less sensitive to \( \beta \)-adrenergic stimulation than the smaller epicardial vessels, suggesting that the responsiveness of the coronary arterial tree to \( \beta \)-receptor agonists has an inverse relation with the size of coronary arteries.\(^5\)

Radioligand binding techniques associated with functional studies in the conscious animal demonstrated the existence of both \( \beta_1 \)- and \( \beta_2 \)-adrenergic receptors in bovine large coronary arteries.\(^6\) Moreover, an equivalent vasodilation of large epicardial arteries after \( \beta_1 \)- or \( \beta_2 \)-adrenergic receptor stimulation was demonstrated, suggesting that both \( \beta_1 \) and \( \beta_2 \)-receptor activation mediates vasodilation in the coronary vascular tree.\(^6\)\(^-\)\(^8\)

It was also suggested that in canine coronary arteries \( \beta \)-adrenergic receptors may mediate endothelium-dependent vasorelaxation. In fact, vasodilation elicited by \( \beta \)-adrenergic receptor agonists was reduced after the removal of the endothelium.\(^9\)\(^,\)\(^10\) However, recent autoradiographic investigations were unable to identify \( \beta \)-adrenergic receptors in the endothelium of the canine left anterior descending coronary artery.\(^11\)

Although \( \beta \)-adrenergic blockade is in widespread clinical use in the treatment of angina pectoris,\(^12\) only limited information is available so far concerning the subtype or the localization of \( \beta \)-adrenergic receptors in human coronary arteries.\(^1\) The main report on this subject described an intimal localization of \( \beta_1 \)-adren-
ergic receptors in sections of small coronary artery branches of the left ventricle papillary muscle. However, no data are available concerning the subtype or the distribution of β-adrenergic receptors in human large epicardial arteries.

The purpose of the present study was to analyze the pharmacological characteristics and the anatomic localization of β-adrenergic receptors in sections of the human right and left coronary arteries and of the anterior interventricular branch using combined in vitro radioreceptor binding and autoradiographic techniques.

Materials and Methods

Chemicals

[^125]Cyanopindolol ([^125]CYP; specific activity, 2,000 Ci/mmol) was purchased from Amersham Radiochemical Centre, UK. CGP 20712A was a product of CIBA-GEIGY, Basel, Switzerland. Both isomers of propranolol and ICI 118,551 were products of Imperial Chemical Industries, UK. GTP was obtained from Boehringer-Mannheim, FRG. Other chemicals of analytical grade were purchased from Sigma Chemical Co., St. Louis, or Merck, Darmstadt, FRG.

Tissue Preparation

One-centimeter right and left coronary arteries and anterior interventricular branch samples were obtained from three subjects who died from car accidents (one female, 13 years old; two males, 17 and 19 years old). The hearts were removed to be transplanted, but when they arrived at the transplantation unit, they were found to be unsuitable for transplantation and were refused, becoming available for the present study. At an internal inspection, indeed, a traumatic tear of the atrial septum (in one specimen) and wide subendocardial hemorrhages in the left ventricle (in the other two cases) were noticed. Italian law does not allow hearts to be removed from donors for any reason but for transplantation, and that accounts for the inevitably small size of the sample available for the present study.

Since the removed specimens were intended to be used for transplantation, they were processed according to the routine protocol. First of all, the inferior vena cava was tied, and the right superior pulmonary vein was cut. The heart was emptied and consequently arrested. The aorta was immediately clamped, and the coronary arteries were perfused with a cold (4°C) hyperkalemic cardioplegic solution. In the meantime, a cold Ringer’s lactate solution was poured into the pericardial sac.

Soon afterward, the heart was removed, put on sterile ice prepared for transplantation, and introduced in a bag containing a cold (4°C) Ringer’s solution. Such a bag was then introduced into two subsequent bags containing cold Ringer’s fluid. These concentric bags were needed to preserve both low temperature and sterility in the inner bag, which was to be opened in the operating theater only.

For every transplant procedure, a cardiac pathologist is always present in the cardiac transplantation unit to receive and immediately sample and freeze the recipient’s heart. In the above-mentioned cases, the pathologist at once sampled the refused, but otherwise normal, donors’ hearts. Three 1-cm-long segments of coronary artery were excised, washed in ice-cold Krebs-Ringer solution (to remove blood and cell debris), reduced into small rings, and embedded in cryoprotectant (OCT compound, Ames, Iowa). The specimens were designated and sampled as follows: 1) right coronary artery: proximal right coronary artery, 1 cm from the coronary ostium; 2) left coronary artery: proximal left main coronary artery, near the exit from the aortic wall; 3) anterior interventricular branch: proximal left anterior descending coronary artery, 1 cm from left coronary trunk bifurcation. OCT blocks were quickly frozen in isopentane that was cooled in liquid nitrogen and stored at −80°C until use. Serial transverse sections were obtained on a microtome cryostat and mounted on gelatin-coated microscope slides.

Binding Experiments

Sections (8 µm thick) were preincubated for 30 minutes at room temperature in Krebs’ buffer of the following composition (mM): NaCl 118.4, KCl 4.7, MgSO4 1.2, CaCl2 1.27, and Na2HPO4 10; the buffer contained 0.1 mM ascorbic acid, 0.1 mM EDTA, 10 µM phenylmethylsulfonyl fluoride, and 0.1 mM GTP. The slides were then incubated in the preincubation buffer but without GTP for 120 minutes at room temperature with increasing concentrations (from 10 to 120 pM) of [^125]I-CYP in the presence or absence of the β1-adrenergic receptor selective antagonist CGP 20712A (100 nM) or the β2-adrenergic receptor selective antagonist ICI 118,551 (70 nM). The standard concentrations of CGP 20712A and of ICI 118,551 were established on the basis of competition binding experiments performed with 10 different concentrations of the two displacers.

The inhibition of binding by selective competing agents was evaluated with the equation developed by Neve et al and subsequently applied by Murphy and Saffitz to autoradiography: B=(B max,1·L)/[L+K d,1·(1+I/K 1)] + (B max,2·L)/[L+K d,2·(1+I/K 2)], where B is the amount of radioligand bound; B max,1 and B max,2 are densities of β1- and β2-adrenergic receptor subtypes, respectively; L is the concentration of radioligand ([^125]I-CYP); K d,1 and K d,2 are the dissociation constants of the radioligand at β1- and β2-adrenergic receptors, respectively; K 1 and K 2 are dissociation constants of the competing agent (CGP 20712A or ICI 118,551) at β1- and β2-adrenergic receptors, respectively; and I is the concentration of the competing agent.

In a series of preliminary experiments, the optimal incubation and washing conditions were assessed (data not shown). Nonspecific binding was defined by the presence in the incubation medium of 1 µM...
Autoradiography

Sections (5 μm thick) processed as described for the above binding studies were air-dried and processed for autoradiography according to the technique proposed by Young and Kuhar.20 Briefly, acid-washed, gelatin-coated coverslips were dipped in nuclear emulsion (L4 diluted 1:1 in distilled water, Ilford, Cheshire, UK) at 40°C and dried in a light-proof box containing P2O5 at room temperature for at least 3 hours. Emulsion-coated coverslips were attached to slides carrying radiolabeled sections with cyanoacrylate glue at one end and a binder clip at the other. Nuclear emulsion was exposed for 2–5 days, the binder clips were removed, and the coverslips were gently lifted from microscope slides at one end. The emulsions were then developed with Kodak D19, fixed with Kodak fixer, and washed in distilled water. Coronary artery sections were stained with toluidine blue, and the coverslips were sealed permanently to the slides. The tissue and overlying silver grains in the emulsion layer were viewed and photographed with a bright-field- and dark-field-equipped Zeiss II (Carl Zeiss, Oberkochen, FRG) photomicroscope.

The density of silver grains developed within the adventitia, media, and intima of the three epicardial arteries examined was assessed on dark-field–observed sections exposed for 3 days to the nuclear emulsion by counting the number of silver grains developed in [125I]CYP autoradiographs, according to the procedure described in an earlier study.21 The silver grains were counted with a Zeiss II dark-field photomicroscope using a planapochromat ×40/1.0 objective and a 2-optimvar to obtain a final magnification of ×400. Counts were made independently by three investigators in 10 consecutive sections of each artery per subject (n=3); the sections were incubated with 70 pM L-propranolol. At the end of incubation, the slides were washed at 37°C17 in the incubation buffer (two times for 15 minutes each) and quickly rinsed in distilled water.

The specificity of [125I]CYP binding to β-adrenergic receptors was assessed by incubating some sections with 70 pM [125I]CYP in the presence of various concentrations of L- or D-propranolol, L- or D-isoprotanol, and L- or D-norpinephrine. Sections used for binding experiments were wiped onto glass fiber filters (model GF-B, Whatman Inc., Clifton, N.J.) and counted in a gamma counter (Pharmacia LKB Biotechnology, Piscataway, N.J.). Representative sections mounted on acid-washed (non–gelatin-coated) slides were sonicated, and the protein content was determined according to Lowry et al19 against a standard of bovine serum albumin.

Figure 1. Graph showing effect of temperature on [125I]cyanopindolol binding to sections of human coronary artery. Sections were incubated with 70 pM radioligand. After selected time intervals, sections were rinsed in cold buffer to remove unbound radioligand, air-dried, and counted in a gamma counter. Each point represents the mean±SD of triplicate determinations.

Figure 2. Graphs showing binding isotherms of [125I]cyanopindolol ([125I]CYP) binding to sections of human right (panel A) and left (panel B) coronary arteries. ○, Total binding; ◻, specific binding; ●, nonspecific binding. Sections were incubated under equilibrium binding conditions at 25°C with increasing concentrations of radioligand (10–120 pM), then rinsed, air-dried, and counted in a gamma counter. Standard deviation bars are indicated for total and nonspecific binding. Each point represents the mean±SD of triplicate determinations.
TABLE 1. $K_d$ and $B_{max}$ Values Obtained in Slide-Mounted Sections of Coronary Arteries of the Three Subjects Investigated

<table>
<thead>
<tr>
<th>Subject 1 (13 years)</th>
<th>$K_d$ (pM)</th>
<th>$B_{max}$ (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCA</td>
<td>34.9±0.2</td>
<td>40.2±2.5</td>
</tr>
<tr>
<td>LCA</td>
<td>35.5±0.5</td>
<td>33.3±2.4*</td>
</tr>
<tr>
<td>AIB</td>
<td>34.9±0.4</td>
<td>24.7±2.7†</td>
</tr>
<tr>
<td>Subject 2 (17 years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCA</td>
<td>34.7±0.4</td>
<td>42.9±1.9</td>
</tr>
<tr>
<td>LCA</td>
<td>35.6±0.3</td>
<td>37.5±1.8*</td>
</tr>
<tr>
<td>AIB</td>
<td>35.3±0.3</td>
<td>26.1±2.5†</td>
</tr>
<tr>
<td>Subject 3 (19 years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCA</td>
<td>34.8±0.3</td>
<td>41.4±2.0</td>
</tr>
<tr>
<td>LCA</td>
<td>35.2±0.4</td>
<td>35.4±2.3*</td>
</tr>
<tr>
<td>AIB</td>
<td>35.2±0.5</td>
<td>26.3±2.9†</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM of six independent isotherms per artery of each subject. RCA, right coronary artery; LCA, left coronary artery; AIB, anterior interventricular branch.

$^*p<0.05$ vs. right coronary artery; $fp<0.01$ vs. right or left coronary artery.

$[^{125}I]$CYP in the presence of 70 nM ICI 118,551 (to label $\beta$-adrenergic receptors), 100 nM CGP 20712A (to label $\beta$-adrenergic receptors), or 1 $\mu$M L-propranolol (to cause nonspecific binding). Measurements were made in a 400-$\mu$m area of each arterial layer as delineated by an image analyzer (Videoplan, Carl Zeiss) connected via a video camera with the microscope.

Data Analysis

All data are expressed as mean±SD unless otherwise specified. Binding isotherm data were transformed according to the method of Scatchard. Simple linear regression was used in determining intersections and slopes in Scatchard plots. Competitor dissociation constant values ($K_d$) were determined according to the method of Cheng and Prusoff.

The statistical significance of differences in the density of $[^{125}I]$CYP binding sites in the three arteries under study was determined with analysis of variance.

Results

$[^{125}I]$CYP was specifically bound by sections of human coronary arteries. The binding was time, temperature (Figure 1), and concentration (Figure 2) dependent. As shown in Figure 1, the plateau in specific binding was reached after 60 minutes of incubation and remained unchanged for the subsequent 90 minutes. Thus, in subsequent binding experiments, a constant incubation time of 90 minutes was chosen. Temperatures of 25°C or 37°C did not cause statistically significant changes in the amount of $[^{125}I]$CYP bound to sections of coronary arteries (Figure 1). A standard 25°C incubation temperature was therefore used in subsequent experiments. Binding isotherms of sections of human coronary arteries incubated with $[^{125}I]$CYP are shown in Figure 2. As can be seen, the apparent $K_d$ values were quite close.

Figure 3. Graphs showing inhibition of specific $[^{125}I]$cyanopindolol binding with increasing concentrations of CGP 20712A (A, a $\beta$-adrenergic receptor antagonist) or ICI 118,551 (B, a $\beta$-adrenergic receptor antagonist) in slide-mounted sections of right coronary artery (panel A) and anterior interventricular branch (panel B). The curves are biphasic and characterized by low pseudo–Hill coefficients. Points are duplicate. Vertical lines indicate standard deviation.
similar in the three arteries under study and averaged \(\sim 35\) pM. Scatchard analysis of isotherms in coronary arteries (six isotherms in sections of each coronary artery from three donors) revealed \(B_{\text{max}}\) values of 41.5 fmol/mg protein for the right coronary artery, 35.4 fmol/mg protein for the left coronary artery \((p<0.05\) versus right coronary artery), and 25.7 fmol/mg protein for the anterior interventricular branch \((p<0.01\) versus right and left coronary arteries). The \(K_d\) and \(B_{\text{max}}\) values per artery obtained in the three different subjects examined are summarized in Table 1.

The binding of \(^{125}\)I]CYP was stereospecific. In fact, as shown in Table 2, L-stereoisomers were from two to three orders of magnitude more effective than D-stereoisomers in displacing \(^{125}\)I]CYP binding from sections of human coronary arteries. The most effective displacer was L-propranolol, followed, in descending order, by L-isoproterenol and L-norepinephrine.

Competition experiments using the highly selective \(\beta_1\)-antagonist CGP 20712A and the highly selective \(\beta_2\)-antagonist ICI 118,551 produced biphasic competition curves in the three coronary arteries examined (Figure 3). From the analysis of these curves, it was established that the optimal concentrations of CGP 20712A and of ICI 118,551 to block \(\beta_1\) and \(\beta_2\)-adrenergic receptor binding sites were 100 and 70 nM, respectively. The same concentrations were reported by other authors\(^{11,13}\) to be optimal in human cardiac tissues and canine coronary arteries.

The proportion of \(\beta_1\) and \(\beta_2\)-adrenergic receptor binding sites in slide-mounted sections of human coronary arteries examined is shown in Table 3. As can be seen, in the right coronary artery, the relative amounts of \(\beta_1\) and \(\beta_2\)-adrenergic receptors were 72% and 28%, respectively; in the left coronary artery, 65% and 35%, respectively; and in the anterior interventricular branch, 40% and 60%, respectively.

**Autoradiography**

Silver grains representing specific \(^{125}\)I]CYP binding sites were observed within the walls of the right

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**Table 3. Percentages of \(\beta_1\) and \(\beta_2\)-Adrenergic Receptor Binding Sites in Slide-Mounted Sections of Human Coronary Arteries**

<table>
<thead>
<tr>
<th>Artery</th>
<th>(n)</th>
<th>(\beta_1)-receptor (%)</th>
<th>(\beta_2)-receptor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right coronary</td>
<td>3</td>
<td>72.4±5.2(^*)</td>
<td>27.6±3.0(^*)</td>
</tr>
<tr>
<td>Left coronary</td>
<td>3</td>
<td>65.4±4.8(^*)</td>
<td>34.6±3.9(^*)</td>
</tr>
<tr>
<td>Anterior interventricular</td>
<td>3</td>
<td>39.7±3.1</td>
<td>60.3±5.2</td>
</tr>
</tbody>
</table>

Values shown are mean±SD from \(n\) experiments. Proportion of \(\beta_1\) and \(\beta_2\)-adrenergic receptors in sections of human epicardial arteries examined were calculated by determining the inhibition of total \(^{125}\)I]cyanopindolol binding by CGP 20712A (100 nM) or ICI 118,551 (70 nM) using the equation reported in "Materials and Methods."

\(^{*}\)p<0.01 vs. anterior interventricular artery.

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**Figure 4.** Light microscope autoradiography showing \(\beta_1\)-adrenergic receptor localization in the human right coronary artery. *a*, Adventitia; *m*, media; *i*, intima; *L*, lumen. Panel A: Dark-field picture of a section incubated with 70 pM \(^{125}\)I]cyanopindolol plus 70 nM ICI 118,551 to block \(\beta_2\)-adrenergic receptors. Silver grains were accumulated primarily within the medial layer. Panel B: Bright-field picture of panel A stained with toluidine blue to verify microanatomic details. Panel C: Dark-field picture of a section adjacent to panel A incubated as above but in the presence of L-propranolol (1 \(\mu\)M) to cause nonspecific binding. Magnification, \(\times 120\).
Panel A: Dark-field picture of a section incubated with 70 nM ICI 118,551 showing the localization of silver grains in the medial layer. Panel B: Bright-field picture of panel A stained with toluidine blue to verify microanatomic details. Panel C: Dark-field picture of a section adjacent to panel A incubated as above but in the presence of I-propranolol (1 μM) to cause nonspecific binding. Magnification, ×140.

Figure 5. Light-microscope autoradiography showing the localization of β₁-adrenergic receptors in the human left coronary artery. a, Adventitia; m, media; i, intima; L, lumen. Panel A: Dark-field picture of a section incubated with 70 pM [³¹²]cyanopindolol plus 70 nM ICI 118,551 showing the localization of silver grains in the medial layer. Panel B: Bright-field picture of panel A stained with toluidine blue to verify microanatomic details. Panel C: Dark-field picture of a section adjacent to panel A incubated as above but in the presence of I-propranolol (1 μM) to cause nonspecific binding. Magnification, ×140.

and left coronary arteries and the anterior interventricular branch (Figures 4–6). The density of silver grains determined by the simultaneous presence in the incubation medium of [¹²⁵I]CYP and 1 μM I-propranolol (nonspecific binding) was substantially lower than the one observed by incubating sections in the presence of [¹²⁵I]CYP alone (Figures 4–6).

Sections incubated with 70 pM [¹²⁵I]CYP (a concentration two times higher than Kᵢ value), in the presence of 100 nM CGP 20712A to label β₂-adrenergic receptors, developed silver grains in the three arterial layers but with a higher density in the adventitia, the adventitial–medial border, and the intima layer (Figure 6). Sections incubated with 70 pM [¹²⁵I]CYP, in the presence of 70 nM ICI 118,551 to label β₁-adrenergic receptors, developed silver grains primarily in the medial layer of human coronary arteries, most likely within smooth muscle (Figures 4 and 5). β₁-Adrenergic receptor binding sites were homogeneously distributed within the entire medial layer of different coronary arteries.

Analysis of the density of silver grains developed after autoradiography is summarized in Figure 7. As can be seen, in agreement with receptor binding data, the highest accumulation of silver grains occurred in the right coronary artery, followed, in descending order, by the left coronary and the anterior communicating artery. The highest accumulation of β₁-adrenergic receptors was noticeable in the adventitia of the anterior communicating artery followed by the left and then the right coronary arteries. The density of intimal silver grains is not significantly different in the three arteries examined (Figure 7).

Discussion

It has been suggested that both circulating and neurally released norepinephrine causes vasodilation of the large coronary arteries by interacting with β₁-adrenergic receptors. However, although no conclusive hypothesis can be drawn as to the significance of β₁-adrenergic receptors in the physiological control of coronary hemodynamics, available evidence suggests an important role for β₁-adrenergic receptors in the control of coronary resistance vessels.

The main data on the pharmacological characterization of large coronary artery β₁-adrenergic receptors were obtained in bovine large coronary arteries by the use of binding techniques and functional studies in conscious animals. As outlined in the introductory section, both nonspecific (isoproterenol) or specific (such as prenarterol, a β₁-agonist, or pirbutrol, a β₂-agonist) β₁-adrenergic receptor agonists mediated vasodilation. This kind of β₁- or β₂-receptor mediated coronary vasodilation was considered independent of changes in cardiac metabolism and therefore linked to the activation of specific β₁-adrenergic receptors in large epicardial arteries. Radioligand experiments demonstrated the existence of both subtypes of β-adrenoceptors in the ratio 1.5:1. The first studies on the autoradiographic localization of β₁-adrenergic receptors in the heart and coronary vasculature were published by Muntz and colleagues in 1984 and 1986. Their work demonstrated, in the dog, a greater density of β-ad-
renergic receptors in intramyocardial coronary resistance vessels compared with large epicardial coronary arteries. This work was completed and expanded by a more recent binding and autoradiographic study on the canine left anterior descending coronary artery; this study demonstrated the coexistence of β₁- and β₂-adrenergic receptors in the proportion 85%:15%. β₁-Adrenergic receptors were found in the smooth muscle of the artery; β₂-adrenergic receptors were located primarily on small nervous trunks of the perivascular connective tissue and on the adventitia. A medial localization of a few β₂-adrenergic receptors was also noticeable.

Only sparse information is now available concerning the β-adrenergic receptor subtypes in human coronary arteries (see Reference 13). This is due to the impossibility, in the past, of obtaining fresh samples of human epicardial arteries for biochemical or functional studies. In that respect, the increasing number of heart transplants in many countries allowed researchers to obtain more human material for their studies. However, when hearts are obtained from patients needing heart transplantation, there is the risk that pharmacological treatments or pathological processes may determine significant modifications of parameters to be evaluated. In our study, we had the opportunity to examine the main epicardial arteries from three healthy patients whose hearts were removed for transplantation. However, just after removal, these hearts were considered to be unsuitable for transplantation due to the presence of traumatic lesions. Hence, the material used in the present study can be considered to be normal fresh samples of coronary arteries. Moreover, the homogeneity in the age of donors and the similarity in the results obtained in the different patients allow us to hypothesize that our small group is rather representative of the distribution and pattern of coronary β-adrenergic receptors. On the other hand, although small in size, due to the extreme difficulty of obtaining fresh normal samples of human heart and/or coronary arteries, our work must be considered as a contribution to the better understanding of the extension and significance of β-adrenergic receptor systems in the coronary circulation in normal conditions.

In the present study, we demonstrated that [125I]CYP was bound to mounted sections of human coronary arteries in a manner consistent with the labeling of β-adrenergic receptors. In fact, the binding of the ligand was saturable, stereospecific, reversible, and displaceable by antagonists and agonists with the rank order of potency expected for β-adrenergic receptors. The use of the β₁-adrenergic receptor antagonist CGP 20712A or the β₂-adrenergic receptor antagonist ICI 118,551 allowed us to identify the subtype of adrenergic receptor present in the coronary arteries investigated. Similarly, as described for porcine, canine, and bovine coronary epicardial arteries, both β₁- and β₂-adrenergic receptors were identified in human large coronary arteries. The relative percentages of β₁- and β₂-adrenergic receptors were similar in the right and in the left coronary arteries; in the anterior interventricular branch, β₂-adrenergic receptors predominate. Moreover, in view of the possible nonabsolute specificity of displacers used for the two subtypes of
β-adrenergic receptors investigated, the different percentages of β₁- and β₂-adrenergic receptors characterized probably reflect the percentages of these sites in general rather than in absolute terms. Of course, the functional significance of the different density of the two subtypes of β-adrenergic receptors in the arteries investigated, if any, should be clarified in future studies.

Autoradiography revealed that β₁-adrenergic receptors predominate in the medial layer of human large coronary arteries, as described similarly in canine anterior descending coronary artery.11 These findings allow us to hypothesize that the β₁-adrenergic receptor stimulation–elicited vasodilatation7,24 is caused by the direct interaction with receptor sites located within vascular smooth muscle. A similar hypothesis could be drawn concerning β₂-adrenergic receptors located in the medial layer. More difficult to suggest, in terms of anatomic distribution, is the meaning of coronary β₂-receptors. They are accumulated primarily within the adventitia, the adventitial–medial border, and the intima. Since the adventitia and the adventitial–medial transitional zone represent the vascular portions where the sympathetic nerve plexus ends,31 these binding sites likely represent prejunctional receptors located on sympathetic endings. Alternative hypotheses are that they represent the so-called “silent receptors,” with the function of reducing the concentration of neurotransmitters available for effector cells,32 or that they may be postjunctural in nature, in some way related to effereent vegetative mechanisms at the adventitial level.33 On the basis of our findings, we are unable to say whether intimal β₂-adrenergic receptors represent endothelial receptors sensitive to circulating catecholamines34 or blood products bound to the coronary artery intimal layer.11

In spite of the unsolved problems concerning the meaning of adventitial and intimal β₂-adrenergic receptor binding sites, the characterization and the localization of β₂-adrenergic receptors in human large coronary arteries may contribute to a better knowledge of the mechanisms involved in the control of coronary circulation in humans.

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KEY WORDS: human coronary arteries • \( \beta \)-adrenergic receptors • \( \beta_2 \)-adrenoceptors • radioreceptor binding • autoradiography
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