Positron Emission Tomography With $^{11}$C CGP-12177 to Assess β-Adrenergic Receptor Concentration in Idiopathic Dilated Cardiomyopathy

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**Background.** Positron emission tomography (PET) with $^{11}$C-labeled CGP-12177 (CGP) has been shown to have the potential to noninvasively measure β-adrenergic receptor concentration in dog heart. The present study was undertaken to evaluate the clinical value of this technique.

**Methods and Results.** Eight normal subjects and 10 patients with heart failure related to an idiopathic cardiomyopathy were studied. Estimation of β-receptor concentration was based on a graphic method applied on myocardial PET time-concentration curves obtained after an intravenous injection of $^{11}$C-CGP followed 30 minutes later by a coinjection of labeled and unlabeled CGP. The clinical tolerance of these injections was good. Left ventricular concentration of β-receptors was decreased in patients compared with controls (3.12±0.51 versus 6.60±1.18 pmol/mL, respectively; $p<0.001$). This 53% decrease agrees with previous in vitro data. In eight of the 10 patients, the β-receptor concentration obtained from PET was compared with the β-receptor density determined on left ventricular endomyocardial biopsy samples by in vitro binding technique using $^3$H-CGP-12177. Results obtained with both techniques were correlated ($r=0.79$, $p=0.019$). Moreover, decreased β-receptor concentration correlated with the β-contractile responsiveness to intracoronary dobutamine infusion ($r=0.83$, $p=0.003$), indicating a direct link between changes in the receptor number and its biological function.

**Conclusions.** PET appears to be a safe and reliable method of assessing in vivo changes in the number of left ventricular β-adrenergic receptor sites of patients with idiopathic cardiomyopathy. (Circulation 1993;87:1169–1178)

**Key Words** • downregulation • dobutamine • CGP-12177 • congestive heart failure • positron emission tomography • idiopathic dilated cardiomyopathy

In the failing human heart, the myocardial responsiveness to β-agonist stimulation is blunted, mainly due to downregulation of β-adrenergic receptors,1-3 uncoupling of β-adrenergic receptors,4 and modification of the functional activity of G protein subunits.5 In congestive heart failure, the alterations of the adrenergic system have been shown to be of interest for pathophysiological understanding, therapy evaluation, and patient management. The clear relation between the degree of changes in the adrenergic system and the severity of heart failure has suggested that measurements of adrenergic disorders may be used as prognostic markers.2-9 In addition, in patients with heart failure, therapeutic agents acting directly or indirectly on the adrenergic system have demonstrated their ability to improve either clinical or hemodynamic conditions as well as diminish the level of adrenergic system impairment.9-13

The in vivo assessment of β-adrenergic pathway in patients with heart failure is mainly based on the determination of β-adrenergic receptor density on endomyocardial biopsy samples or on the measurement of cardiac responsiveness to β-agonist stimulation. Previous results from our laboratory have shown in the nonfailing dog myocardium that in vivo and noninvasive quantification of the β-adrenergic receptor concentration by positron emission tomography (PET) was an achievable goal.14 This method is based on the use of $^{11}$C-CGP-12177 (CGP) as a ligand and on a graphic analysis of PET time–concentration curves obtained with a specific protocol. The protocol involves two injections, one of which includes administration of unlabeled ligand to occupy a significant proportion of receptor sites.14 CGP appears to be the most suitable ligand because it is a very potent and hydrophilic antagonist with low nonspecific binding on membranes and slight cellular uptake.15 CGP enables the exploration of plasma membrane receptors, which are thought
to be functionally coupled to the adenyl cyclase–G protein complex. Nevertheless, as a potent antagonist of β-receptors, CGP may have adverse effects when injected intravenously into patients with heart failure. However, previous animal experiments have shown that only low doses of unlabeled ligand were required for our PET protocol, suggesting that this technique could be safely used for clinical investigation.14

The aim of the present study was to evaluate the ability of PET to measure changes in the number of β-receptor sites in human heart. Normal subjects and patients with congestive heart failure related to an idiopathic cardiomyopathy were investigated. In patients, the left ventricle β-receptor concentration measured by PET was compared with the left ventricle β-receptor density determined in vitro on endomyocardial biopsy samples with a binding assay using 3H-CGP-12177. Moreover, the consequences of the decrease in concentration of β-receptors on the myocardial contractile function were examined in patients by comparing PET data with the left ventricular contractile responsiveness to intracoronary dobutamine infusion.

See p 1412

Methods

Study Population

Patients. Ten patients (nine men and one woman; mean age, 46±9 years) with at least one episode of acute congestive heart failure related to an idiopathic dilated cardiomyopathy were included in the study after fulfilling the criteria of symptoms of congestive heart failure grade II–IV in the functional classification of the New York Heart Association for more than 6 months, radionuclide left ventricular ejection fraction of less than 40% (mean, 22±7%), sinus rhythm, and absence of asthma and atrioventricular block. Idiopathic cardiomyopathy was considered present when the coronary arteriogram did not show any significant stenosis (no narrowing of more than 50% of the lumen artery) and when no other recognized etiology was evident.

At least 1 week before entering the study, all patients were clinically stable with diuretics and vasodilators but not with inotropic or β-blocking agents.

Control subjects. Eight normal male subjects (mean age, 41±5 years) were studied to determine control values for plasma catecholamine concentration and PET-measured myocardial β-receptor concentration. These control subjects showed no sign of cardiac disease at clinical, ECG, and echocardiographic examinations and were taking no medication. These subjects had no contraindication to the use of β-blocking agents.

The research protocol was approved by the Henri Mondor University Hospital Ethics Committee, and each subject gave written informed consent.

PET Imaging Protocol

Synthesis of 11C-CGP. The pharmacologically active enantiomer (2S)-CGP-12177 [(2S)-4-(3-t-butylamino-2-hydroxypropoxy)-benzimidazol-2-one] was synthesized and labeled with 11C, the synthesis being accomplished from (2S)-3-tosylxoy-1,2-propanediol acetonide. The enantiomeric excess was more than 98%. 11C-CGP-12177, in the S form, was obtained with a specific radioactivity of 350–1,200 mCi/μmol. A more detailed description of the synthesis can be found elsewhere.16

Data acquisition. Subjects were positioned in the TTV03 time-of-flight PET scanner (LETI, CEA, Grenoble, France). This instrument allows acquisition of seven cross-sectional images, 12 mm apart, with a 7-mm in-plane resolution on a reconstructed image using a modified Hanning window function. The axial resolution is 9 mm for a direct plane and 7 mm for a cross plane.17 Transmission scans were obtained with a retractable 67Ge ring source and used for subsequent attenuation correction of the emission scans. Emission data were acquired in list-mode over 70 minutes. The ECG was monitored continuously during the examination as well as 30 minutes before and 60 minutes after the PET scan. Blood pressure was measured before injection and every 2 minutes after each injection.

Experimental protocol. From the amount of 11C-CGP synthesized, two doses of tracer were obtained and put into two different syringes. The first injection consisted of a trace dose D1* of 11C-CGP, ranging from 4 to 6 mCi, and was administered at the beginning of the experiment (T0). The second injection consisted of the injection of a mixture of labeled and unlabeled CGP and was administered 30 minutes later (T1) as a slow bolus over 1 minute. The corresponding dose of labeled ligand (D1*) ranged from 4 to 6 mCi. The corresponding dose of unlabeled ligand (D1) ranged, according to weight, from 7.5 to 20 μg in controls and from 5 to 12 μg in patients.

Thirty images were reconstructed (eight 1-minute images, 16 2-minute images, and six 5-minute images).

Graphic determination of the receptor concentration.

The graphic method, described elsewhere,14 is based on this two-injection protocol and is justified by the properties of the CGP kinetics. In particular, a few minutes after the injection of the labeled ligand, the myocardial time–concentration curve takes the shape of a plateau. The receptor concentration is estimated by using two experimental myocardial concentration values calculated from the PET time–concentration curve (Figure 1). C0* represents the intercept on the concentration axis of the straight line (logarithmic scale) corresponding to the plateau following the first injection. C1* represents the difference between the concentration at 30 minutes, extrapolated from the straight line obtained after the second injection, and the concentration measured just before this second injection. The proposed graphic method estimates the receptor concentration using five values: the two measured concentrations C1* and C0* and the three doses D1*, D1, and D0, which are the known masses of labeled (*) and unlabeled CGP injected at times T0 and T1.

The concentration of receptors available for binding (B′max) is the solution of the following nonlinear equation:

\[
[B'_{\text{max}} - C^*(T_1 - \varepsilon)] \left[ 1 - \exp \left( \frac{D_1^* - D_1}{D_1^* \log \frac{B'_{\text{max}} - C^*}{B'_{\text{max}}}} \right) \right] C^* = \frac{D_1}{D_1^*} = 0
\]

where C0*(T1 − ε) is the concentration of the labeled CGP just before the second injection.
PET data analysis. The graphic model used is based on measurements of the myocardial concentrations $C_0^{*}$ and $C_1^{*}$ of the labeled ligand obtained from PET tissue concentration curves and resulting from the two injections of the labeled ligand (at masses $D_0^{*}$ and $D_1^{*}$, respectively). Time–activity–concentration curves were measured in regions of interest encompassing the left ventricular myocardium, manually drawn on static 20-minute images of two or three consecutive slices obtained 10 minutes after the first $^{11}$C-CGP injection. $^{11}$C-CGP concentration, expressed in pmol/mL, was deduced from the $^{11}$C activity concentration after correction for $^{11}$C decay and after division by specific radioactivity. The specific radioactivity used to obtain $^{11}$C-CGP concentration during the entire experiment was that measured at time $T_0$ because $^{11}$C-CGP injected at times $T_1$ and $T_2$ was produced by the same synthesis. Moreover, the amount of unlabeled ligand administered through the coinjection was taken into account separately when computing $B_{max}'$, using a second set of coupled differential equations.14 Data were corrected for partial volume effect using echographic measurements of left ventricular wall thickness and a recovery factor measured experimentally on a heart phantom.14,18

Plasma Norepinephrine Determination

Before the PET study was started, venous blood samples were drawn at baseline, after a 30-minute resting period with the subjects in the supine position. Plasma norepinephrine concentrations were determined by radioenzymatic assay.18

Myocardial β-Adrenergic Contractile Responsiveness

Hemodynamic measurements. Patients underwent cardiac catheterization with intracoronary dobutamine infusion within the week before CPG PET imaging. The technique used was similar to that described by Colucci et al.20 Patients fasted at least 12 hours before hemodynamic examination. Local anesthesia was achieved with 1% xylocaine. A 7F Swan-Ganz thermodilution catheter (Edwards Laboratories, Irvine, Calif.) was placed into the pulmonary artery via a femoral vein to determine the cardiac output. A 5F micromanometer-tipped catheter (Millar Industries, Houston, Tex.) was introduced into the left ventricle through a femoral artery. A 5F sheath was placed through the other femoral artery to introduce a 5F L-4 Judkins left coronary catheter into the main left coronary artery for drug infusion. A side-arm sheath (Cordis Laboratories, Roden, The Netherlands) was used to monitor arterial pressure. Pressures were measured using Gould P50 pressure transducers coupled to pressure modules and to a Gould TA 2000 multichannel recorder (Gould Electronics, Ballainvilliers, France). Heart rate was recorded continuously.

Left ventricular end-diastolic pressure, left ventricular systolic pressure, mean right atrial pressure (RAP), mean arterial pressure (MAP), pulmonary-capillary wedge pressure, and the first derivative of the left ventricular pressure (peak positive LV dP/dt) were obtained by electronic integration. All pressures were monitored continuously during the procedure. Cardiac output (CO, L/min) and cardiac index (CI=CO/body surface area; L/min/m²) were determined by the thermodilution method using a bedside Edwards 9520-A computer (Edwards Laboratories, Irvine, Calif.). Each CO value was calculated using the mean value of at least three measurements. Systemic vascular resistance (SVR) was calculated as SVR=(MAP–RAP)/CI.

Intracoronary dobutamine infusion. A 5% dextrose in water solution was previously infused into the main left coronary artery for 15 minutes at a rate of 60 mL/hr. This infusion rate was used for all subsequent dobutamine infusions. Repeated measurements were made during this time period to ensure hemodynamic stability and then used as baseline values. Dobutamine diluted in a 5% dextrose in water solution was consecutively administered for 5-minute periods at incremental infusion rates of 25, 50, 100, 200, and 400 μg/min using an infusion pump (Vial-Medical, Grenoble, France). The increment of infusion rate was stopped if the heart rate increased by more than 10%. The maximal response was taken as the maximal increase in peak positive LV dP/dt.
with no change in heart rate by more than 10%. The net increase in peak positive LV dP/dt (Δpeak positive LV dP/dt) was calculated as the difference between peak positive LV dP/dt at maximal intracoronary dobutamine infusion and that at baseline. ΔPeak positive LV dP/dt was taken as an index of the maximal β-adrenergic contractile response to dobutamine.

All patients received heparin during catheterization. No adverse effects, especially arrhythmias, were observed during dobutamine infusion, and no complication occurred as the result of this procedure.

In Vitro Measurement of β-Adrenergic Receptor Density

Endomyocardial biopsy. In eight of the 10 patients, left ventricular endomyocardial biopsy samples were obtained during the cardiac catheterization procedure, before dobutamine infusion. After removal, samples immediately were placed into liquid nitrogen.

Membrane preparation. Tissue weight ranged from 2.7 to 15.5 mg (mean weight, 7.2±3.8 mg). After defrosting, the biopsy specimens were finely dissected and minced with scissors in 250 μl of ice-cold buffer (50 mM Tris, 10 mM MgCl₂, pH 7.4); the final buffer volume was adjusted to 1 mL. For each sample, membranes were prepared by homogenization—first with a Polytron (PCU-Kinematic, Gmbh-Bioblock, Lucerne, Switzerland; one burst of 5 seconds at the setting of 7) and then with a glass-glass homogenizer, twice, by hand. To have a trivial loss of initial endomyocardial tissue, each homogenization step was followed by a careful rinsing with additional buffer. Finally, 1 mL of ice-cold 2.5 M KCl was added to extract contractile proteins so that a final volume of 5 mL was obtained. After stirring at 4°C for 15 minutes, the suspension was centrifuged at 40,000g for 20 minutes. The supernatant was removed by mild aspiration, and the pellet of the crude membrane preparation was resuspended (by detachment from the tube wall with a small glass stick) and carefully homogenized by hand in 2–3 mL of buffer. According to the initial weight of the biopsy specimens, the final concentration of these suspensions ranged from 14.6 to 78.6 μg protein/mL buffer (mean, 34.4±20.5 μg protein/mL). Protein measurements were performed by Lowry’s method, adapted by Hartree using bovine serum albumin as standard. The membrane homogenate preparation was immediately followed by the receptor binding assay, without storage.

Receptor assay. 3H-CGP was used to determine the density of β-adrenergic receptors. 3H-CGP, purchased from NEN (Du Pont, Boston, Mass.), had a specific activity of 54 Ci/mmol. The assay buffer was the same as the Tris-MgCl₂ buffer used for membrane preparation. In four to eight tubes (depending on the amount of protein present in each suspension), 300 μL of membrane homogenates was prepared to a final volume of 2 mL. Increasing concentrations of 3H-CGP (from 0.2 to 3 nM) were added. The incubation conditions (37°C for 60 minutes) ensured that equilibrium had been reached between the receptors and the radioligand. The reaction was terminated by rapid vacuum filtration through Whatman GF/C filters; filters were washed with a 15-mL excess of ice-cold buffer. These filters were then dried and placed into 5 mL scintillation fluid (Insta-Gel, Packard). A liquid scintillation counter was used to determine the sample radioactivity (Packard SL 2000, 80% efficiency). Nonspecific binding was determined in the presence of 3 μM of propanol and averaged 10–15% of the total. Maximum density (Bmax) and apparent affinity (Kp) of binding sites were assessed in each individual experiment using a nonlinear least squares regression program.

Statistical Analysis

All values are expressed as mean±SD. For statistical analysis, Student’s t tests for paired and unpaired data were used. Correlation coefficients, assuming a linear regression, were calculated for paired variables. A value of p<0.05 was considered statistically significant.

Results

In Vivo Binding With PET

Figure 2 compares the 20-minute images obtained 10 minutes after CGP injection in a patient and in a control subject. Figure 3 shows time–concentration curves recorded in corresponding regions of interest for the same subjects.

In controls, no significant changes in heart rate or blood pressure occurred as a result of the unlabeled CGP injection, nor were any other adverse effects observed. Left ventricular myocardium concentration of β-adrenergic receptors was estimated by the graphic analysis to be 6.61±1.18 pmol/mL. Individual values are listed in Table 1.

The mean age of patients did not differ from that of controls. The mean left ventricular wall thickness was not different in patients compared with controls (9±2 versus 10±3 mm, p=NS). In patients, heart rate decreased after CGP injection (97±8 at baseline versus 81±10 beats per minute, p=0.046). Blood pressure did not change. Two patients showed a transient and minor dyspnea. β-Receptor concentration of the left ventricular myocardium was decreased by 53% in patients compared with controls (3.12±0.51 versus 6.60±1.18 pmol/mL, respectively; p<0.001). Individual data are listed in Table 2. Figure 4 shows no overlap between individual values of controls and patients. In patients, diminished β-receptor concentration in left ventricular myocardium was correlated with decreased left ventricular ejection fraction (r=0.74, p=0.014; see Figure 5).

Plasma Norepinephrine Concentration

Norepinephrine concentration was higher in patients than in controls (1.455±0.878 and 0.668±0.180 ng/mL,

<table>
<thead>
<tr>
<th>Subject</th>
<th>B'max (PET) (pmol/mL)</th>
<th>[NE] (ng/mL)</th>
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<tr>
<td>1</td>
<td>7.144</td>
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</tr>
<tr>
<td>2</td>
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<tr>
<td>6</td>
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<td>0.535</td>
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<tr>
<td>7</td>
<td>7.468</td>
<td>0.552</td>
</tr>
<tr>
<td>8</td>
<td>5.895</td>
<td>0.354</td>
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Mean value 6.610±1.189 0.668±0.180
FIGURE 2. Myocardial distribution of $^{11}$C-CGP obtained by positron emission tomography (20-minute static image) in a normal subject (top panel) and in a patient with severe left ventricular dysfunction related to an idiopathic dilated cardiomyopathy (bottom panel). A, left ventricular anterior wall; S, interventricular septum; L, left ventricular lateral wall. In the patient, the myocardial uptake of labeled CGP appears to be high, contrasting with decreased concentration of available receptor sites ($B'_\text{max}$) found when using our graphic method. This is due to the fact that the images are normalized, with white and yellow corresponding to pixels of highest activity.

respectively; $p=0.025$). In patients, norepinephrine concentration did not correlate with myocardial $\beta$-receptor concentration.

**In Vitro Binding**

Mean $\beta$-receptor density ($B_{\text{max}}$) measured by the in vitro binding technique was $56.8 \pm 15.9$ fmol/mg protein. Individual values are given in Table 2. $K_d$ was $0.53 \pm 0.20$ nmol/L.

As shown in Figure 6, the $\beta$-adrenergic receptor concentration measured by PET was correlated with the $\beta$-adrenergic receptor density measured by in vitro binding using $^3$H-CGP ($r=0.79, p=0.019$).

**Intracoronary Dobutamine Infusion in Patients**

The maximum increase in peak positive LV $dP/dt$ with no change in heart rate was obtained with a 200-$\mu$g/min dose of dobutamine for eight patients and of 100 $\mu$g/min for the other two. Dobutamine infusion resulted in an increase in cardiac index, a decrease in both pulmonary capillary wedge pressure, and left ventricular end-diastolic pressure as well as a slight decrease in systemic vascular resistance (Table 3). $\Delta$Peak positive LV $dP/dt$ was $647 \pm 471$ mm Hg/sec.

As shown in Figure 7, the contractile responsiveness to intracoronary dobutamine infusion, assessed as the net increase in LV $dP/dt$ during the drug infusion, correlated with myocardial $\beta$-receptor concentration measured by PET ($r=0.83, p=0.002$).

**Discussion**

This is the first report that demonstrates that PET can assess the myocardial $\beta$-receptor concentration in the failing human heart.

The hydrophilic compound $^{11}$C-CGP has been previously shown to meet the criteria needed to characterize the specific binding of a ligand to its receptor: high affinity, saturability, and stereospecificity.$^{15,23}$ One of
the main advantages of CGP is that it binds to surface membrane receptors, the majority of which presumably are functionally coupled to adenylyl cyclase. CGP offers the opportunity to detect variations in the number of external receptors without any change in the total number of receptor sites. However, despite a slight $\beta_1$-selectivity, this ligand binds to both $\beta_1$- and $\beta_2$-receptors and cannot directly detect selective variations in one of these receptor subtypes.

The $\beta$-adrenergic receptor concentration can be determined by PET with a graphic method requiring a specific protocol that consists of an injection of labeled CGP followed, 30 minutes later, by a coinjection of both labeled and unlabeled CGP. This method is based on hypotheses that have been supported by data obtained from dog experiments. First, the myocardial time-concentration curve reaches a plateau rapidly after the injection of labeled ligand and remains constant unless a displacement experiment is performed. This suggests that during the plateau, the measured PET concentration mainly corresponds to the bound radioligand concentration. Second, a displacement experiment showed that the dissociation rate is sufficiently low, so the consequences of these dissociations can be neglected for a few minutes. It should be pointed out that using the same coinjection protocol, time-concentration curves obtained from human examinations were similar to those observed in dog experiments.

This method has several advantages. Based on the hypothesis of the proportionality between the injected dose and the free ligand concentration, it was shown that it is unnecessary to measure the input function and thus to take arterial blood samples and measures of metabolites. Moreover, the influence of myocardial blood flow can be neglected, assuming that myocardial blood flow remains constant between the two CGP injections. To quantify myocardial receptor concentration, it has been shown that at least two injections of

**FIGURE 3.** Experimental time-concentration curves measured in the left ventricular region of interest of a normal subject (top panel) and of a patient (bottom panel) when using the coinjection protocol.
ligand are necessary, one of which includes a dose of unlabeled ligand, to occupy a significant proportion of available receptor sites. For clinical investigation, a coinjection protocol represents the only alternative to determine β-adrenergic receptor concentration without using the displacement procedure, which involves higher amounts of unlabeled CGP, potentially deleterious. Indeed, as previously shown, only low doses of unlabeled CGP appear to be necessary to quantify β-receptor concentration. Moreover the coinjection was well tolerated in the present patient population.

On the other hand, this method has limitations of its own. Contrary to the more complicated PET method previously used to characterize muscarinic-cholinergic receptors in the dog heart, the affinity constant \( K_d \) cannot be identified using the present method because the free ligand concentration remains unknown. The short period of \(^{11}\text{C}\) and the fact that the displacement experiment may induce adverse effects do not allow the measurement of the undisplaceable fraction that corresponds to a nonspecific binding. However, previous studies in vitro have shown that the nonspecific binding is a small proportion of total binding. In the present study, nonspecific binding was low in patients when determined in vitro using tritiated CGP (10–15%). Nevertheless, the proportion of nonspecific binding may be different under in vivo conditions. Previous findings suggest that this is not the case. When performing in vitro binding with tritiated CGP on intact myocytes (enzymatically dissociated cardiac myocytes), that is, under conditions that resemble in vivo more than in vitro situations, the proportion of nonspecific binding was found to be even lower (5–10%). Moreover, in dog, we have estimated in vivo using PET the proportion of nonspecific binding as low as 10%, using a long PET experiment and a displacement procedure at high doses of cold CGP (unpublished data). Another limitation of the present method is that PET ability for absolute quantification of tissue labeled ligand concentration can be altered by changes in left ventricular wall thickness and impaired wall motion. This potential cause of error will be overcome only with the emergence of new PET systems with higher sensitivity and better spatial resolution. To account for changes in left ventricular wall thickness, a recovery coefficient based on echocardiographic measurements is currently used. In the present study, mean left ventricular thickness value was similar for patients and controls. Potential errors due to impaired wall motion were not accounted for in the present study, and this might explain the positive correlation found between left ventricular ejection fraction and \( B_{\text{max}} \). However, the decrease in left ventricular ejection fraction has been previously related to the level of downregulation as assessed in vitro. Moreover, our PET results were compared with in vitro measurements of β-receptor density.

The present study shows the value of PET for measuring myocardial β-receptor concentration. Left ventricle β-receptor concentration of patients with heart failure was decreased by 53% in comparison with controls. A clear-cut difference was observed between individual values of the two groups. β-Adrenergic receptor concentration correlated with left ventricular ejection fraction. These findings agree with previous data obtained using the in vitro binding technique. In
the present study, the β-receptor density of patients was determined in parallel on left ventricle endomyocardial biopsy samples by in vitro binding technique with tritiated CGP. The mean $B_{\text{max}}$ found in the present study was higher than that found by Böhm et al.\(^\text{31}\) in explanted heart from patients with dilated cardiomyopathy using in vitro binding with tritiated CGP ($B_{\text{max}}$ of 34±5.5 fmol/mg of protein). This discrepancy may be explained by the different clinical status of the two patient populations. In the present study, patients were clinically stable in functional class II–IV of the New York Heart Association, whereas in the other study, all patients were in functional class IV of the New York Heart Association and underwent heart transplantation. The difference between the mean values of β-adrenergic receptor density obtained in the two studies is consistent with the fact that β-receptors downregulate in proportion to the severity of heart failure. In the present study, patients with the most severe impairment of cardiac function had individual values of β-adrenergic receptor density close to the mean value found by Böhm et al. Finally, the present study showed that results of in vitro and in vivo bindings, both using CGP as a ligand, were correlated despite expected differences between these two techniques. Indeed, in vitro binding is performed on images from at least two large slices of left ventricular myocardium, including endocardial and epicardial layers. Moreover, results of in vitro binding are normalized by myocardial protein concentration, whereas results of in vivo binding are normalized by myocardial volume unit.

This study also examined the relation between the decrease in left ventricular β-receptor concentration assessed by PET and the main biological effect mediated by these receptors. Human myocardium relies mainly on β-receptors to augment contractility and appears to have no or few “spare” receptors.\(^\text{20,33}\) The present data show a positive correlation between the β-receptor concentration and the contractile responsiveness to intracoronary infusion of dobutamine. This correlation suggests that the downregulation of β-receptors is a main factor of the β-adrenergic desensitization in patients with idiopathic dilated cardiomyopathy. This is consistent with other findings that showed a reduced basal, Gpp(NH)p, and isoproterenol-stimulated adenyl cyclase activity but an unchanged forskoline-stim-

**FIGURE 5.** Plot of correlation between the radionuclide left ventricular ejection fraction (LVEF) and the left ventricular concentration of β-adrenergic receptors ($B'_{\text{max}}$) assessed by positron emission tomography.

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<thead>
<tr>
<th>TABLE 3. Hemodynamic Effects of Intracoronary Dobutamine Infusion</th>
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<td><strong>Baseline</strong></td>
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<tr>
<td>Heart rate (bpm)</td>
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<td>Systematic vascular resistance (IU)</td>
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<td>Cardiac index (L/min/m²)</td>
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<td>Left ventricular end-diastolic pressure (mm Hg)</td>
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<td>Peak positive left ventricular dP/dt (mm Hg/sec)</td>
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*<p<0.05, †p<0.005.
lated adenyl cyclase, suggesting that reduced isoproterenol-stimulated adenyl cyclase is due to reduced β-adrenergic receptor density rather than increased inhibitory subunit of G protein.34 Because β2-receptor density is not altered in patients with heart failure,33,35 the decrease in β-receptor concentration found by PET would be mainly due to the downregulation of externalized β2-receptors. However, this approach has potential limitations. Although dobutamine was administered into the left main coronary artery to minimize the drug’s effect on heart rate and systemic vascular resistance, a slight decrease of the latter was observed, which may interfere with the contractile response. Radioligand binding and physiological data suggest that dobutamine is a nonselective agonist of adrenergic receptors, interacting mainly with β1-adrenergic receptors but also with α1- and β2-receptors, which can, in the failing myocardium, mediate a positive inotropic response. However, α1-receptor density is not altered or slightly increased in heart failure,36 and β2-receptors do not downregulate but are only partially uncoupled.35 In fact, although dobutamine infusion and in vivo binding with CGP potentially explore both β1- and β2-receptor pathways, it is likely that the alterations detected by the two techniques in patients with heart failure are predominantly related to the impaired β1-pathway function.

Finally, PET appears to be a reliable and accurate means of assessing in vivo modifications of the number of β-adrenergic receptor sites in human heart. Moreover, despite the necessity of injecting nonnegligible amounts of unlabeled CGP, this method can be safely used in patients with heart failure, even in those severely diseased, provided they are clinically stable at the time of examination. This noninvasive technique offers the possibility of repeating measurements of β-receptor concentrations during follow-up, enabling the evaluation of the effects of therapy on the number of β-receptor sites.

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