In vivo quantification of pulmonary β-adrenoceptor density in humans with (S)-[^{11}C]CGP-12177 and PET

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UEKI, J., C. G. RHODES, J. M. B. HUGHES, R. DE SILVA, D. C. LEFROY, P. W. IND, F. QING, F. BRADY, S. K. LUTHRA, C. J. STEEL, S. L. WATERS, A. A. LAMMERTSMA, P. G. CAMICI, AND T. JONES. *In vivo quantification of pulmonary β-adrenoceptor density in humans with (S)-[^{11}C]CGP-12177 and PET.* J. Appl. Physiol. 75(2): 559–565, 1993.—The in vivo regional distribution of pulmonary β-adrenoceptors was imaged and quantified in humans with the hydrophilic β-adrenoceptor antagonist (S)-CGP-12177 labeled with carbon-11 {(S)-[^{11}C]CGP-12177} and positron emission tomography (PET). Six normal male volunteers and eight patients with hypertrophic cardiomyopathy were studied. PET scanning consisted of transmission (tissue density), C^{15}O (blood volume), and (S)-[^{11}C]CGP-12177 (β-adrenoceptor) emission scans. High-specific-activity (S)-[^{11}C]-CGP-12177 (7.1 ± 2.0 μg, 6.5 ± 2.1 GBq/μmol) was given intravenously followed by a low-specific-activity (S)-[^{11}C]CGP-12177 injection (34.0 ± 4.8 μg, 2.3 ± 0.8 GBq/μmol). Binding capacity (Bmax) was calculated in each region of interest as picomoles per gram by normalizing it to the local extravascular tissue density. In normal subjects, average Bmax for all regions of interest was 14.8 ± 1.6 (SD) pmol/g, which is similar to previously reported in vitro values. In both groups there were no differences in β-adrenoceptor density between peripheral and central regions nor between right and left lungs. In patients with hypertrophic cardiomyopathy, extravascular tissue density was 24% higher than in normal subjects; Bmax per milliliter thoracic volume was correspondingly higher but was not different from that in normal subjects when expressed per gram tissue (15.8 ± 2.6 pmol/g). These data suggest that in vivo β-adrenoceptor density may be quantifiable in humans with the use of PET. This should offer a means to study physiological regulation through repeat measurements.

binding capacity; pulmonary extravascular density; pulmonary blood volume; hypertrophic cardiomyopathy

PULMONARY β-ADRENOCEPTORS are not only of fundamental importance in the regulation of airway caliber, especially in asthma, but also may play a role in the modulation of ganglionic transmission, surfactant production, alveolar-capillary fluid balance, mast cell and other inflammatory cell functions, and glandular secretion (17). Furthermore, changes in β-adrenoceptor function have been demonstrated in asthma, cystic fibrosis, and emphysema (6). However, the question of up- and downregulation of the pulmonary β-adrenoceptor in vivo has yet to be resolved. This has been mainly because of the lack of suitable methods that are repeatable for studying the physiological regulation of pulmonary β-adrenoceptor density serially in a single individual in vivo.

Indirect assessment of the pharmacological regulation of the β-adrenoceptor density in lung tissue has used receptor assay of the freely available mononuclear leukocytes thought, possibly, to reflect the properties of the pulmonary β-adrenoceptor (1). However, Hauck et al. (10) found no reduction of β-adrenoceptor density in resected lung tissue from patients who had been treated with terbutaline and showed downregulation of their mononuclear leukocyte receptor pool. Although receptors are frequently studied in surgically resected or postmortem lung tissue (5, 10, 22), the operative procedure and premortem condition will influence the results and the measurements cannot be repeated.

Positron emission tomography (PET) provides quantitative information about regional lung structure, mechanical and metabolic function, and tissue pharmacokinetics. With the use of PET, techniques have been developed to measure regional pulmonary extravascular density (20), blood volume (20), glucose metabolic rate (18), and antimicrobial drug transport (31). When used with suitable radiolabeled ligands, PET can also be used to measure receptor density in vivo (9, 25).

The β-adrenoceptor antagonist CGP-12177 has a number of properties that render it an ideal ligand for quantifying β-adrenoceptor density. Because of its high hydrophilicity and high affinity, CGP-12177 binds selectively to cell surface β-adrenoceptors in intact cells without binding to internalized β-adrenoceptors (23). Although (R,S)-CGP-12177 has been previously labeled with carbon-11 (21), the S enantiomer of CGP-12177 was proven better for PET studies because of its higher affinity, resulting in a greater specific signal (14). In a previous study from our group, (S)-[^{11}C]CGP-12177 was used to measure pulmonary β-adrenoceptor density in the dog lung (19).

The aim of the present investigation was to assess the feasibility of quantifying in vivo pulmonary β-adrenoceptor density noninvasively in humans by studying a group of normal human volunteers with (S)-[^{11}C]CGP-12177 and PET. In addition, pulmonary β-adrenoceptor density was calculated from data recorded in a group of patients with hypertrophic cardiomyopathy (HCM), previously studied in our institute, to determine whether the β-adrenoceptor downregulation demonstrated in the

myocardium of these patients (15) was a localized phenomenon.

METHODS

Preparation of (S)-[^{11}C]CGP-12177

For labeling (*S*)-CGP-12177 [(*S*)-(3′-*tert*-butylamino-2′-hydroxypropoxy)-benzimidazol-2-one] with the short-lived positron-emitting radionuclide carbon-11 (half time = 20.4 min), the diamino precursor (*S*)-[1-(2,3-diaminophenoxy)]-3′-(*N*-*tert*-butylamino)propan-2′-ol was synthesized asymmetrically in three steps from 2,3-dinitrophenol and the chiral auxiliary (*S*)-glycidyl-3-nitrobenzene sulfonate. Reaction of the diamino precursor with [^{11}C]phosgene provided (*S*)-[^{11}C]CGP-12177 in >99% chemical and radiochemical purity. Product identity was confirmed by mass spectrometry (electron impact and chemical ionization modes), and the enantiometric purity (>95% *S* enantiomer) was determined by circular dichroism (3).

Study Population

Subjects consisted of six healthy normal male volunteers [mean age 26 yr (range 21–34 yr)] and eight patients with HCM [mean age 37 yr (range 20–51 yr), 7 males]. All subjects underwent preliminary screening, which included a full clinical history and examination. All normal subjects were asymptomatic, with no history of taking β-agonists or β-blocking drugs, and were normal on physical examination. The diagnosis of patients with HCM had previously been made on the basis of clinical and echocardiographic criteria, and none had ever taken β-blocking drugs. All medication was stopped 3 days before the scan. Patients were clinically stable with normal spirometry (mean vital capacity, 100.0 ± 14.9% predicted; forced expiratory volume in 1 s, 94.1 ± 13.9% predicted). Chest films showed no abnormal pulmonary shadowing.

All subjects gave written informed consent to the protocol, which was approved by the Hammersmith Hospital Research Ethics Committee and the United Kingdom Administration of Radioactive Substances Advisory Committee.

Study Protocol

PET scans were performed using an ECAT 931–08/12, 15-plane positron tomograph (Siemens/CTI, Knoxville, TN). Each plane had a slice thickness of 6.6 mm full width at half maximum, and the total thickness of the lung imaged in the transaxial direction was 10.8 cm. The transaxial spatial resolution was 8.4 mm full width at half maximum. PET scanning consisted of *1*) transmission, *2*) C^{15}O emission, and *3*) (*S*)-[^{11}C]CGP-12177 dynamic emission scans to measure pulmonary extravascular density, blood volume, and β-adrenoceptor density, respectively. The subject lay supine on the bed. A venous cannula was inserted into a forearm vein for blood sampling, and a second venous cannula was inserted into the other arm for the tracer infusions. Arterial pressure was measured by cuff sphygmomanometry, and an electrocardiogram was monitored every 10 min throughout the study.

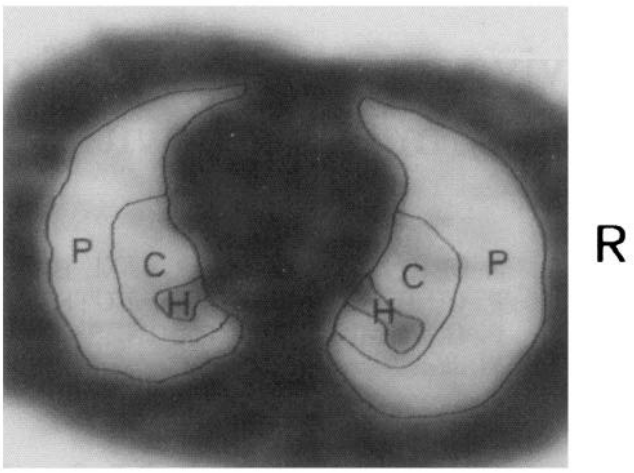

FIG. 1. Image of transmission scan of thorax. Regions of interest were drawn on this image to divide lungs into peripheral (P) and central (C) portions. Hilar structures (H) were excluded from central lung portions. R, right lung; L, left lung.

Transmission scan. The scan was performed in the transmission mode for 20 min during the exposure of a set of ring sources containing the long-lived positron emitter germanium/gallium-68, which encircled the subject. These data were used for attenuation correction of all subsequent emission data and also provided images of the lung density distribution [vascular plus extravascular, g/ml (g tissue/ml thoracic volume); Fig. 1].

C^{15}O emission scan. To obtain pulmonary vascular blood density [g/ml (g blood/ml thoracic volume)], a 6-min emission scan was performed 5 min after the start of a 4-min inhalation of C^{15}O in air (Fig. 2*B*). C^{15}O was administered at a concentration of 3 MBq/ml and a flow of 500 ml/min. C^{15}O combines with hemoglobin to form [^{15}O]carboxyhemoglobin in the red blood cells of the lung capillaries. Blood samples were taken 0, 2, 4, and 6 min after the start of C^{15}O scan to relate vascular radioactivity to the equilibrium images of the C^{15}O distribution, thereby allowing the calculation of regional pulmonary blood volume [ml/ml (ml blood/ml thoracic volume)] to be used later in the calculation of receptor density. Values of blood volume were converted to vascular density (20) by multiplying by 1.06 (whole blood density). Quantitative images of pulmonary extravascular tissue density (g/ml) were then calculated by subtracting the vascular density distribution from the normalized transmission images as previously described (20) (Fig. 2*A*).

(S)-[^{11}C]CGP-12177 dynamic emission scan. Measurement of pulmonary β-adrenoceptor density was performed using a modification of the double-injection method of Delforge et al. (8). A high-specific-activity (*S*)-[^{11}C]CGP-12177 preparation (7.1 ± 2.0 μg, 155.1 ± 32.5 MBq; specific activity 6.5 ± 2.1 GBq/μmol; n = 14) was first given intravenously over 2 min followed 30 min later by a second injection of (*S*)-[^{11}C]CGP-12177 with a lower specific activity (34.0 ± 4.8 μg, 273.4 ± 77.9 MBq; specific activity 2.3 ± 0.8 GBq/μmol). Dynamic emission

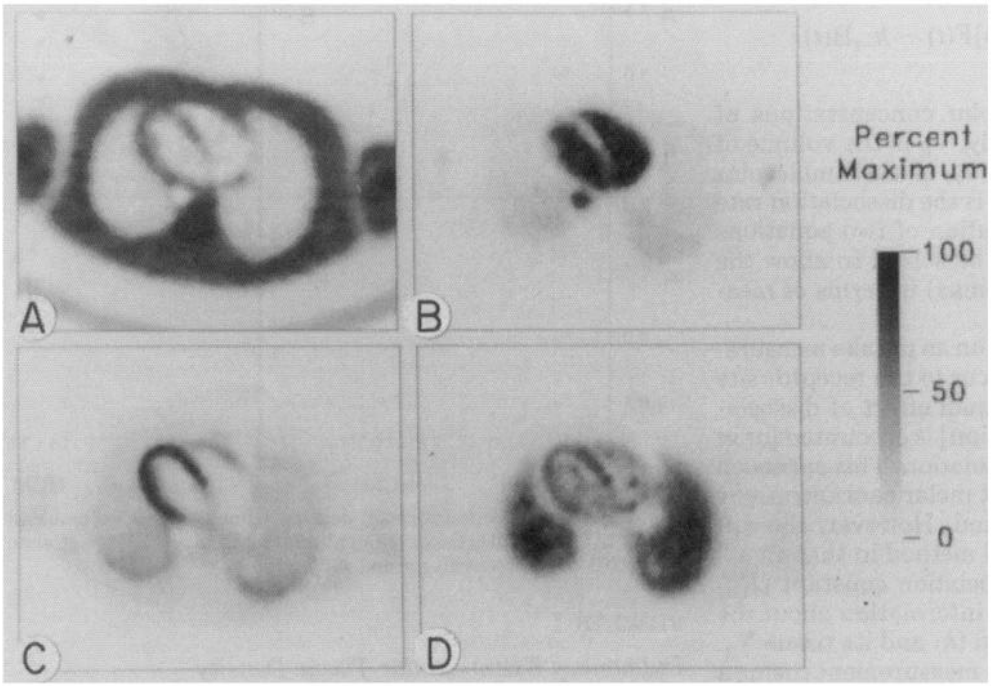

FIG. 2. Images of pulmonary extravascular tissue density (*A*), blood volume (*B*), β-adrenoceptor binding per ml thorax (*C*), and β-adrenoceptor binding per g tissue (*D*) obtained from study on normal volunteer comprising single injection of (*S*)-[^{11}C]CGP-12177 at high specific activity. *C* was obtained by adding dynamic time frame images recorded between 20 and 80 min.

scanning comprising 45 time frames was started at the time of the first (*S*)-[^{11}C]CGP-12177 injection and continued for 60 min (Fig. 2*C*).

Calculation of β-Adrenoceptor Density

To determine the topography of pulmonary β-adrenoceptors, regions of interest (ROIs) were drawn on the transmission images to divide both right and left lungs into peripheral and central portions, halfway between the hilum and chest wall (Fig. 1). In the 15 planes scanned, peripheral portions were selected from the second plane above the diaphragm (the most caudal plane) to the highest plane in the lung (the most cranial). The most caudal plane for the ROI of the central portion was at the level of the inferior pulmonary vein. To generate pulmonary tissue tracer time-activity curves, ROIs were projected onto the dynamic (*S*)-[^{11}C]CGP-12177 images. The summed tracer activity in serial lung planes (craniocaudal) was averaged and plotted against time (Fig. 3). The extravascular tissue tracer time-activity curve was obtained by subtracting the pulmonary vascular (*S*)-[^{11}C]CGP-12177 time-activity curve [calculated from the C^{15}O blood volume data and (*S*)-[^{11}C]CGP-12177 activity in the venous blood samples] from the tissue (*S*)-[^{11}C]CGP-12177 time-activity curve. A lung-to-peripheral blood hematocrit ratio of unity was assumed. A graphic approach derived from Delforge et al. (8) was used to calculate the binding capacity (Bmax) of pulmonary β-adrenoceptors in each ROI. The total amount of cold ligand in both injections was taken into account. This technique was further modified to express Bmax as picomoles per gram (pmol/g tissue) by normalizing it to the local value of extravascular tissue density (Fig. 2*D*).

Theory of the graphic method. This approach, which has been previously described in detail for the measurement of receptor density in the myocardium by Delforge et al. (8), relies on a difference in the kinetic behavior of the radioactive ligand when injected under conditions of medium and high specific activity [i.e., low and medium molar amounts of (*S*)-CGP-12177] and is based on the following differential equation

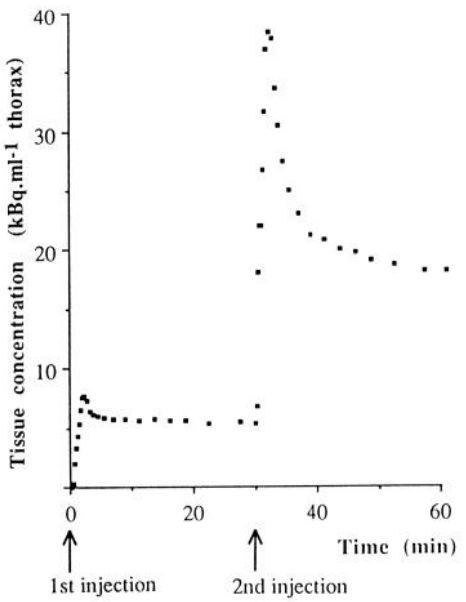

FIG. 3. Typical tissue tracer time-activity curve obtained in peripheral lung in normal subject by double injection of (*S*)-[^{11}C]CGP-12177 at high (1st) and medium (2nd) specific activities. Slope of curve after both injections is characterized by fast and slow component relating to washout of free (either vascular or extravascular) and bound tracer, respectively. More cold ligand is administered in 2nd injection, which results in decreased level of tracer binding because of additional competition for free receptors. This is manifested by a reduction in height of slow component (relative to initial peak) and increase in its slope.

$$\frac{dB(t)}{dt} = \frac{k_{+1}}{V_R}[Bmax - B(t)]F(t) - k_{-1}B(t)$$

where $B(t)$ and $F(t)$ are the molar concentrations of bound and free ligand, respectively; V_R is the volume of reaction for free ligand in tissue; k_{+1} is the bimolecular association rate constant; and k_{-1} is the dissociation rate constant. This allows the formulation of two equations (one for each injection) that can be solved to allow the calculation of receptor density (Bmax) in terms of measurable quantities.

The method is essentially based on an uptake measurement where association of the tracer to the receptor site dominates the kinetics and the small effect of dissociation [the term $k_{-1}B(t)$ in the equation] is accounted for in the analysis by exponential extrapolation. This approach is analogous to the use of different molar concentrations of ligand in the Scatchard method. However, the approach differs from the Scatchard method in that an assessment of the equilibrium dissociation constant (K_D) can be made only with additional information about the efflux rate constant for free ligand (k) and its tissue V_R. In addition, a saturation clearance measurement (using a high-affinity agonist or antagonist) would be needed to measure the ligand's k_{-1}.

Statistical Analysis

All values are expressed as means ± SD. In each group, unpaired t tests were used to compare the values of pulmonary extravascular tissue volume, blood volume, and β-adrenoceptor density between right and left lungs and between peripheral and central lung portions. Unpaired t tests were also carried out for the comparison of the above parameters between normal volunteers and patients. $P < 0.05$ was considered statistically significant.

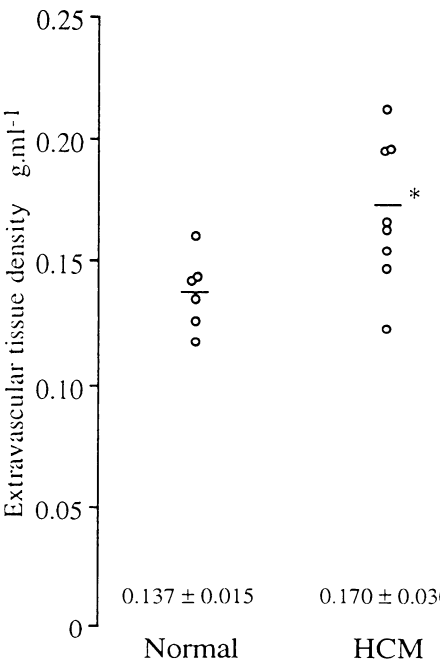

FIG. 4. Mean pulmonary extravascular tissue density in normal subjects and hypertrophic cardiomyopathy (HCM) patients. * $P < 0.05$ compared with normal subjects.

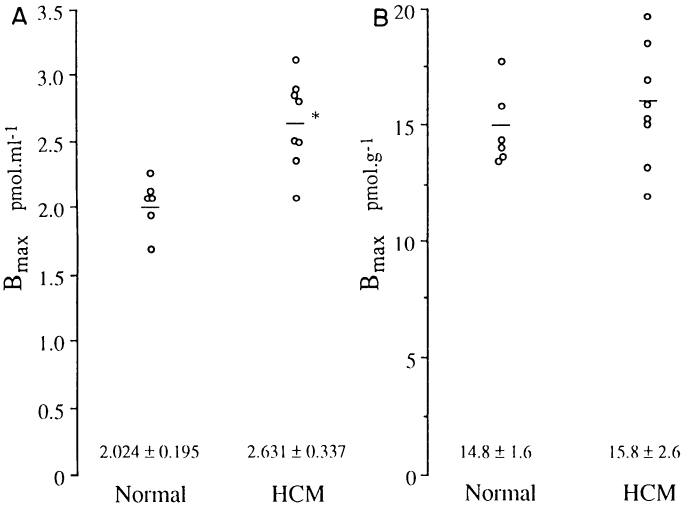

FIG. 5. Mean β-adrenoceptor density (Bmax) given in pmol/ml thorax (A) and pmol/g tissue (B) in normal subjects and HCM patients. * $P < 0.01$ compared with normal subjects.

RESULTS

Pulmonary Extravascular Tissue Density

The mean extravascular tissue density was higher in HCM patients than in normal subjects [0.170 ± 0.030 vs. 0.137 ± 0.015 g/ml (g tissue/ml thoracic volume), P = 0.03, Fig. 4]. In both groups, the mean extravascular tissue density was higher in the left than in the right lung (0.156 ± 0.025 vs. 0.126 ± 0.014 g/ml in normal subjects, P = 0.03; 0.194 ± 0.027 vs. 0.157 ± 0.034 g/ml in HCM patients, P = 0.03), but there were no significant peripheral-central differences (0.139 ± 0.015 vs. 0.131 ± 0.018 g/ml in normal subjects; 0.170 ± 0.030 vs. 0.167 ± 0.032 g/ml in HCM patients).

Pulmonary Blood Volume

There was no significant difference in the mean pulmonary blood volume between normal subjects and HCM patients [0.153 ± 0.027 vs. 0.155 ± 0.029 ml/ml (ml blood/ml thoracic volume)]. In both groups, the mean pulmonary blood volume was higher in the central than in the peripheral portion (0.189 ± 0.033 vs. 0.144 ± 0.023 ml/ml in normal subjects, P = 0.02; 0.190 ± 0.037 vs. 0.145 ± 0.029 ml/ml in HCM patients, P = 0.02), but there were no significant right-left differences (0.153 ± 0.027 vs. 0.154 ± 0.028 ml/ml in normal subjects; 0.155 ± 0.031 vs. 0.158 ± 0.028 ml/ml in HCM patients).

β-Adrenoceptor Density

The tissue tracer time-activity curve from the peripheral lung of a normal subject is shown in Fig. 5. The mean pulmonary β-adrenoceptor density was higher in HCM patients than in normal subjects [2.631 ± 0.337 vs. 2.024 ± 0.195 pmol/ml (pmol/ml thoracic volume), P = 0.002], but there was no significant difference when normalized to the corresponding value of extravascular tissue density [15.8 ± 2.6 vs. 14.8 ± 1.6 pmol/g (pmol/g tissue) (Fig. 5)]. There were no differences in β-adrenoceptor density between peripheral and central regions in either group of subjects [14.6 ± 1.6 vs. 15.9 ± 3.4 pmol/g

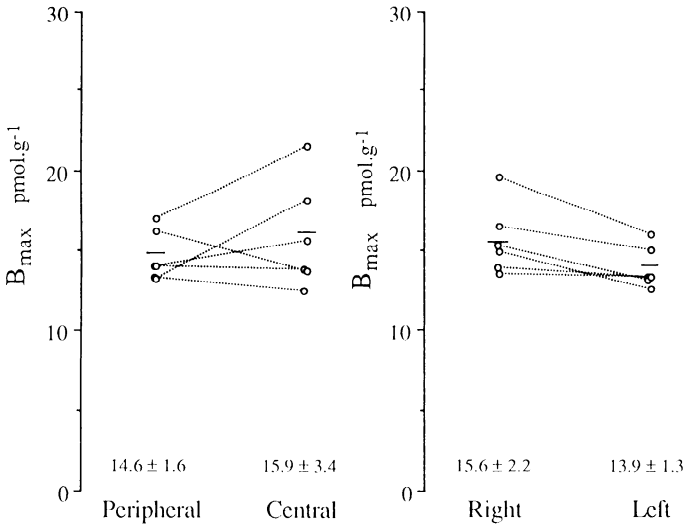

FIG. 6. Regional pulmonary β-adrenoceptor Bmax in peripheral and central lung portions and right and left lungs in normal subjects.

in normal subjects (Fig. 6); 16.3 ± 2.3 vs. 14.7 ± 2.6 pmol/g in HCM patients]. Similarly, there were no differences between right and left lungs [15.6 ± 2.2 vs. 13.9 ± 1.3 pmol/g in normal subjects (Fig. 6) compared with 16.6 ± 3.2 vs. 14.8 ± 1.9 pmol/g in HCM patients].

DISCUSSION

Previous investigation of the pulmonary β-adrenoceptor density in humans has relied on in vitro receptor density measurements of circulating mononuclear leukocytes or assay of pulmonary tissue obtained postmortem or at operation. These approaches are unsatisfactory because the behavior of the mononuclear leukocyte β-adrenoceptor does not appear to follow that of the lung (10) and because tissue sampling only offers a single measurement in time. This does not lend itself to pharmacological studies that require repeat determinations. Furthermore, the physiological status of the lung tissue at the time of sampling is unknown. For these reasons, an in vivo technique was adopted using (*S*)-[^{11}C]CGP-12177 and PET to quantify pulmonary β-adrenoceptor density noninvasively in humans.

β-Adrenoceptor Density in Normal Subjects

The average Bmax for all ROIs measured by PET was 14.8 ± 1.6 pmol/g (n = 6). Bmax for β-adrenergic receptors has been measured in human lobes removed at thoracotomy for carcinoma of the lung (5, 10). Using ^{125}I-cyanopindolol (ICYP), Carstairs et al. (5) studied the binding characteristics of 16-mm-thick sections of peripheral lung and membranes prepared from the tissue. Bmax was 12.6 ± 0.9 (SE) pmol/g lung tissue (n = 9) for the sections and 9.5 ± 0.6 pmol/g (n = 3) for the membrane preparations (assuming the protein fraction of lung tissue is 10%). Hauck et al. (10), using ICYP, studied a similar group of patients; the mean value for their lung membrane preparation was 23.5 ± 2.6 pmol/g (n = 10). In eight patients who had received a β-agonist (terbutaline, 0.5 mg sc 2–6 times) preoperatively, Bmax was 20.4 ± 2.0 pmol/g (n = 8). This difference was not significant. The close agreement with our noninvasive measurements is remarkable.

There are larger bronchi and more bronchial smooth muscle in the central lung portion, but our findings suggest that no significant differences in β-adrenoceptor density exist between central and peripheral lung areas (Fig. 6). Furthermore, no significant right-left differences were observed (Fig. 6). Bmax was consistent within each subject.

β-Adrenoreceptor Density in HCM Patients

Pulmonary data from HCM patients were available from an on-going cardiological study of myocardial β-adrenoceptor density using (*S*)-[^{11}C]CGP-12177 and PET in our unit (15). Although myocardial receptor density was reduced in both hypertrophied and nonhypertrophied heart tissue compared with that in a normal control group (15), there was, as we have shown in this study, no downregulation of pulmonary β-adrenoceptors. The pulmonary Bmax per milliliter of thorax was high, but receptor density was normal when expressed per gram of tissue (Fig. 6). Interestingly, pulmonary extravascular tissue density was raised in the HCM patients (Fig. 4). A number of explanations could account for these observations. The most probable would be that cellular proliferation and vascular hypertrophy were present, reflecting the effect of chronic elevation of pulmonary venous pressure, as indicated by increased left ventricular end-diastolic pressure typical of these patients. However, if this increase in extravascular tissue density had been due to edema fluid or interstitial fibrosis, the result would have been to underestimate Bmax per gram of tissue and mask a real increase in receptor density of pulmonary tissue in the HCM patients. An alternative explanation for the increased values of extravascular tissue density and Bmax per milliliter of thorax could be that there was a degree of hypoinflation of the lung. This, however, would still result in normal values of Bmax per gram of tissue. Our data suggest that the downregulation of β-adrenoreceptors seen globally in the myocardium of HCM patients (15) may be a regional phenomenon occurring only in the heart.

Measurements of Extravascular Density and Pulmonary Blood Volume

The values of pulmonary extravascular density in the normal volunteers are similar to those published previously that made use of a similar technique (4, 20). The increase in tissue density of HCM patients is in keeping with similar findings that used PET in patients with chronic heart failure (30). The increase in tissue density in the left lung compared with the right lung in both normal subjects and in HCM patients can probably be attributed to compression of the left lower lobe by the heart in the supine position (28).

Regional blood volume in normal volunteers was similar to previously published values (4, 20) and was not different in the HCM patients. A previous PET study in patients with chronic heart failure found a reduction in peripheral pulmonary vascular volume (30), but the clin-

ical status of the HCM patients in this series was considerably better.

Methodological Considerations

Choice of β-adrenoreceptor ligand. CGP-12177 is a potent β-adrenergic blocker with high affinity (estimations of the K_D of the *S* enantiomer range from 0.15 to 0.4 nM; Refs. 2, 26) and is hydrophilic, as evidenced by its phosphate buffer saline-to-octanol partition coefficient of 3:1. This ratio contrasts with more lipophilic ligands such as ICYP (1:18) and dihydroalprenolol (DHA; 1:10; Ref. 24). Studies of *S* and *R* enantiomers show that the binding is stereoselective (2). Nonspecific binding of (*R,S*)-CGP-12177 to intact glioma cells has been shown to be low (10%; Ref. 24). CGP-12177 is not taken up by cells, unlike lipophilic ligands, indicating that it binds only to cell surface receptors (23). Comparison of [^{3}H]-CGP-12177 and [^{3}H]DHA binding to mononuclear leukocytes has shown that β-adrenoreceptors desensitized by incubation with isoproterenol lose the ability to bind CGP-12177 while retaining DHA binding (7).

[^{3}H]-CGP-12177 has been injected into rats and tissue radioactivity estimated in postmortem samples with the use of *S* and *R,S* compounds (14, 27). Specificity of the binding of CGP-12177 to β-adrenoreceptors was confirmed by the ability to block or displace the ligand from lung tissue with the β-adrenergic antagonist propranolol (14, 27). In lung tissue, nonspecific binding of the *S* enantiomer varied between 4 and 6%, as determined by the tissue-to-plasma ratios of 170, 6.3, and 9.6, obtained after high-specific-activity injections of [^{3}H]-CGP-12177 and blocking doses of CGP-12177 and propranolol, respectively. The lung uptake index for [^{3}H]CGP-12177 at 10 min (defined as the tissue ligand concentration normalized to injected activity and body weight) was found to be 2 and 17 after excess propranolol and high-specific-activity injection, respectively (14, 27). With the coinjection of (*S*)-[^{3}H]CGP-12177 and varying quantities of unlabeled (*R,S*)-CGP-12177 or propranolol, in vivo saturation curves were constructed and a Bmax of 45 pmol/g lung tissue (wet wt) was obtained (14). This value is similar to that obtained in rat lung with ICYP (40.6 pmol/g; Ref. 29). These studies suggested that CGP-12177 labeled with a positron emitter (^{11}C) would be a suitable ligand for measuring with PET the in vivo β-adrenoceptor density in human lungs.

Pulmonary (11) and cardiac (12) β-adrenergic receptors have been imaged previously with ^{125}I- or ^{131}I-hydroxypindolol in experimental animals with a gamma camera. Nonspecific uptake with this tracer, however, was ~50%, and quantification of binding was not possible. Measurements of myocardial β-adrenoceptors with (*R,S*)-[^{11}C]CGP-12177 and PET have been reported by Delforge et al. (8) in intact dogs. They focused their attention on myocardial β-adrenoceptors and did not report measurements in the lung. However, the performance of the racemate is less than ideal; lung uptake of (*R,S*)-[^{3}H]CGP-12177 was 60%, and lung-to-plasma ratios were only 35% of those obtained with (*S*)-[^{3}H]CGP-12177 (14). Therefore, chiral separation was performed (3).

In vivo metabolism of (S)-[^{11}C]CGP-12177. Measurements using high-performance liquid chromatography analysis have shown that >97% of the radioactivity in plasma ≤60 min after intravenous injection in dogs and humans is unchanged (*S*)-[^{11}C]CGP-12177 (13, 16). In the rat, however, the amount of unchanged ligand has been shown to vary between levels >95% (13) at 30 min and 70% (16, 27) at 60 min. Detectable levels of metabolites were not found in lung tissue taken from rats and dogs (13). In contrast, Delforge et al. (8), using (*R,S*)-[^{11}C]CGP-12177 and thin-layer chromatography in dogs, reported that 5 min after intravenous injection most of the radioactivity in plasma was associated with a compound that was not CGP-12177, presumably a metabolite. These differences are currently being investigated. The absence of significant metabolism in humans is important because it minimizes the degree of nonspecific binding in tissue.

Method for calculating Bmax. In this paper, we used a modification of the graphic method described by Delforge et al. (8) to calculate Bmax for β-adrenergic receptors from PET data. Sequential injections of the radioligand at low and medium molar content (4–11 vs. 28–42 μg, respectively) allow simultaneous equations to be used to calculate receptor density (see METHODS and Ref. 8). A minor aspect of the modification of the technique is that a subtraction of vascular activity is made to the tissue time-activity curve. This relies on a blood volume measurement (using C^{15}O) and blood sampling from a peripheral vein. The sampled blood activity is assumed to equal that in the pulmonary circulation (the approximately equal admixture of small-vessel mixed-venous and arterial blood seen within the lung ROI). To the extent that this subtraction is made, at the earliest, 3 min after the end of the tracer infusion when vascular concentrations have fallen to low levels, substitution of peripheral venous for arterial blood sampling should not introduce significant errors.

Regarding information about K_D, this was unobtainable for a number of reasons. Calculation of K_D requires values for the model parameters k, V_R, and k_{-1}, none of which are known. The possibility of measuring k_{-1} exists but relies on the administration of saturating quantities of a β-antagonist (e.g., propranolol) to displace (*S*)-[^{11}C]-CGP-12177 from lung tissue in vivo. Although this has been achieved in the anesthetized dog (19) where pindolol was given over a short period of time (0.17 mg/kg over 10–20 s), the measurement is complicated by a large increase in the circulating plasma levels of (*S*)-[^{11}C]CGP-12177 (7-fold in the study quoted), which in itself increases nonspecific tissue uptake. In humans, the opportunity to give such large amounts of β-blocker may exist (0.3 mg/kg of propranolol iv), but the duration of such an infusion would have to be much longer (~20 min), which would reduce peak concentrations. Thus K_D or a related parameter was not determined.

In conclusion, we present a means to investigate in vivo pulmonary β-adrenoreceptors in humans. The method is noninvasive and can be repeated after therapeutic interventions, making it possible to study the pharmacological regulation of β-adrenoreceptors in both normal and diseased lungs.

We are grateful to the director and staff of the Medical Research Council (MRC) Cyclotron Unit for the use of their facility, to Claire Taylor and Andreanna Williams for their assistance in performing the PET studies, and to Allen and Hanbury, Ltd. for their support.

F. Qing is the recipient of a British Lung Foundation grant. R. de Silva is the recipient of a MRC Postgraduate Studentship from King's College, London.

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Received 28 October 1992; accepted in final form 5 April 1993.

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