In Vivo Quantification of Human Pulmonary Beta-Adrenoceptor Density Using PET: Comparison with In Vitro Radioligand Binding

Feng Qing, Christopher G. Rhodes, Michael J. Hayes, Thomas Krausz, S. William Fountain, Terry Jones and J.M.B. Hughes
MRC Clinical Sciences Center, Cyclotron Unit, and Departments of Medicine (Respiratory Division) and Histopathology, Royal Postgraduate Medical School, Hammersmith Hospital, London, United Kingdom; Thoracic Surgical Unit, Harefield Hospital, Harefield, Middlesex, United Kingdom

A new method has recently been developed to quantify pulmonary beta-adrenergic receptors in vivo using PET. This study used in vitro radioligand binding assay (RLBA) as the gold standard to validate in vivo PET measurements. **Methods:** Five male patients with lung cancer aged 57 yr (range 42–67 yr) were studied. PET scanning was performed the day before thoracotomy to determine regional pulmonary beta-receptor density. RLBA was carried out on cell membranes prepared from specimens of lung tissue obtained at the thoracotomy to measure beta-receptor density in vitro. In both cases, the hydrophilic nonselective beta-antagonist radioligand (S)-CGP-12177 was used. For PET studies, this was labeled with ^{11}C and for RLBA with ^{3}H. **Results:** In the PET study, beta-receptor density (B_{max}) was 9.43 ± 1.32 pmole g^{-1} tissue. In the RLBA study, B_{max} was 99.0 ± 15.5 fmole mg^{-1} protein, equivalent to 9.90 ± 1.55 pmole g^{-1} tissue. These values are in good agreement with previously reported in vitro measurements on human lung membranes using ^{125}I-iodocyanopindolol. A correlation was found between beta-adrenergic density obtained using PET and beta-adrenergic density obtained using RLBA ($r = 0.92$; $p < 0.05$). **Conclusion:** The results support the use of PET as a new method for imaging and quantifying pulmonary beta-adrenergic receptors in vivo, opening the way for studies of physiological and pharmacological regulation of beta-adrenergic receptors through noninvasive serial measurements.

Key Words: beta-adrenergic receptors; PET; radioligand binding; CGP-12177

J Nucl Med 1996; 37:1275–1281

In humans, beta-adrenoceptors are widely distributed throughout the lung, with more than 70% of them being of the β_2 subtype. On airway smooth muscle, all beta-receptors are of the β_2 subpopulation (*1*). In addition to bronchial smooth muscle relaxation, beta-receptor stimulation modulates many other functions, e.g., surfactant production, mucous and serous gland secretion, ciliary beating, chloride transport across the epithelium, acetylcholine release from parasympathetic nerve terminals and the inhibition of mediator release from mast and other inflammatory cells.

Although changes in beta-receptor function have been demonstrated in chronic obstructive lung disease and cystic fibrosis (*2*), and although beta-agonist drugs have been used widely to treat asthma, beta-receptors have not been quantified directly in the human lung in vivo until recently (*3*), mainly due to a lack of suitable noninvasive methods. Previous investigations of the pulmonary adrenergic system in man have relied upon the in vitro measurement of beta-receptor density of circulating lymphocytes (*4,5*). This approach assumes that lymphocytes reflect the beta-receptor status of lung, or assays of lung tissue obtained postmortem (*6*) or at thoracotomy (*7–9*). Lymphocyte receptors, however, may not necessarily mirror those of the lung (*8–10*). Moreover, the premortem condition (high level of endogenous cortisol and catecholamines) or premortem treatment (possible high dose of corticosteroids and beta-agonists) may well influence the results, and serial measurements on tissue samples, which are necessary for the study of the physiological and pharmacological regulation of receptors, are not feasible.

Early efforts to image intrathoracic beta-receptors in animal models using gamma cameras and iodine-labeled radioligands were only partially successful (*11,12*). Because nonspecific binding was high (>40%), quantification of binding capacity in vivo was not possible. The first satisfactory study to identify beta-receptors noninvasively was the measurement of myocardial beta-receptor density in the dog (*13*), which was achieved in the early 1990s using PET and a hydrophilic ligand (RS)-CGP-12177 labeled with ^{11}C. Subsequently, the active (S) enantiomer of CGP-12177, which is 80 times more potent than the (R enantiomer), was successfully produced by asymmetric synthesis (*14*) to improve the signal-to-noise ratio. The original method was then modified (*3*) to make this PET technique suitable for quantifying beta-adrenoceptors in vivo in the human lung.

Radioligand binding is the method of choice for the in vitro identification and quantification of beta-adrenergic receptors. The purpose of the present study was to employ this classic method to assess the accuracy of PET measurements and to examine the relationship between beta-receptor density (B_{max}) derived from dynamic in vivo PET data and radioligand binding carried out under equilibrium conditions in vitro using the same ligand [(S)-CGP-12177] in the same subjects.

MATERIALS AND METHODS

Patients

Five male patients (aged 57 ± 9 yr) who were scheduled to undergo lung resection for bronchogenic carcinoma were investigated. Their peak flow rates prior to the PET scan were 348 (range 160–480) 1. min^{-1}. These are approximately 60% of normal, which is not unusual in lung cancer patients. None of the patients took beta-agonists, beta-antagonists or any other drugs that could have interfered with the sympathetic nerve system. A common approach to general anesthesia was followed in all patients. Induction was with thiopentone and anesthesia was provided by N_2O-enflurane and nondepolarizing muscle relaxants. All patients 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.

Received May 22, 1995; revision accepted Sep. 21, 1995.
For correspondence or reprints contact: J.M.B. Hughes, DM, Department of Medicine (Respiratory Division), Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, United Kingdom.

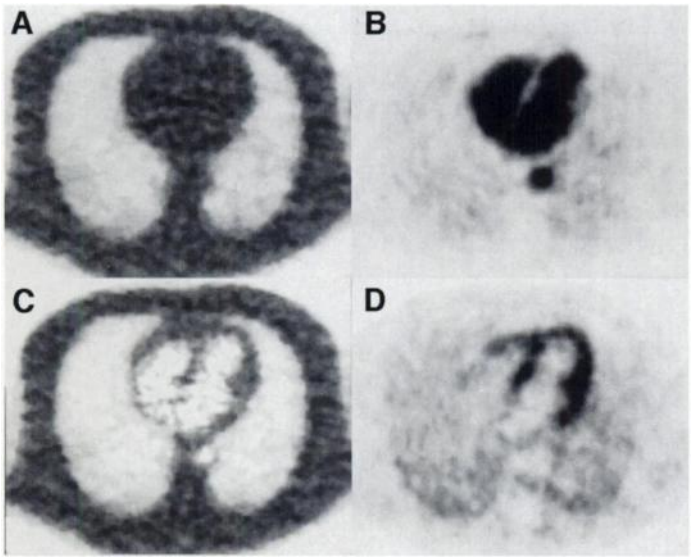

FIGURE 1. Representative images of total lung density (A), regional pulmonary blood volume (B), extravascular tissue density (C) and beta-adrenoceptor binding per milliliter thorax (D) obtained from Patient 3. Image D was obtained by adding the dynamic time frame images recorded between 10 and 30 min after the first injection of (S)-[^{11}C]CGP-12177.

In Vivo Measurement of Beta-Receptors

PET Scanning. PET scans were performed using a 15-plane positron camera with a slice thickness of 6.6 mm FWHM; the total thickness of the transaxial lung sections was 10.8 cm. PET scanning consisted of transmission, $C^{15}O$ emission and (S)-[^{11}C]CGP-12177 dynamic emission scans. The patients lay in the supine position. A venous cannula was inserted into a forearm vein for blood sampling and a second venous cannula was inserted into the other arm for tracer infusion. Arterial blood pressure and electrocardiograms were monitored throughout the study.

Transmission Scan. The scan information was recorded for a 20-min period during the exposure of a ring source of positron-emitting ^{68}Ge/^{68}Ga which encircles the subject. These data were used for attenuation correction of all subsequent emission data and also provided images of the lung density distribution, D_{total} (vascular plus extravascular, g ml^{-1} [g tissue per ml thoracic volume]) (Fig. 1A).

Oxygen-15-Carbon Monoxide Emission Scan. To obtain pulmonary blood volume V_B (ml ml^{-1} [ml blood per ml thoracic volume]), a 6-min emission scan was performed 5 min after the start of a 4-min inhalation of ^{15}O-labeled carbon monoxide ($C^{15}O$) in air. $C^{15}O$ was administered at a concentration of 3 MBq ml^{-1} and at a flow rate of 500 ml min^{-1}. $C^{15}O$ 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 the $C^{15}O$ scan to relate vascular radioactivity to the equilibrium images of the $C^{15}O$ distribution, thereby allowing the calculation of regional pulmonary blood volume (Fig. 1B) to be used later in the calculation of receptor density. Regional blood density values D_B (g blood ml^{-1} thorax) were obtained by multiplying V_B by 1.06 (whole blood density). Quantitative images of pulmonary extravascular tissue density D_{ev} (g ml^{-1}) (Fig. 1C) were then calculated by subtracting D_B from the normalized transmission scan or D_{total} as previously described (*15*).

Carbon-11-CGP-12177 Dynamic Emission Scan. (S)-CGP-12177 [(3′-tert-butylamino-2′-hydroxypropoxy)-benzimidazol-2-one] was asymmetrically synthesized and labeled with the short-lived positron-emitting radionuclide ^{11}C ($T_{½}$ = 20.4 min) on site (*14*). (S)-[^{11}C]CGP-12177 produced by this method exceeded 99% chemical and radiochemical purity. Measurement of pulmonary beta-adrenoceptor density was performed using a modification of the double injection method of Delforge et al. (*13*). A high specific activity (S)-[^{11}C]CGP-12177 preparation (175.3 ± 13.3 MBq (S)-[^{11}C]CGP-12177 in 4.6 ± 0.2 μg cold (S)-CGP-12177) was given intravenously over 2 min, followed 30 min later by a second injection of (S)-[^{11}C]CGP-12177 with a lower specific activity (347.6 ± 27.0 MBq (S)-[^{11}C]CGP-12177 in 25.2 ± 1.0 μg cold (S)-CGP-12177). Cold CGP-12177 was added to the original preparation (where necessary) prior to dividing the sample for the two injections, in order to provide the required cold CGP-12177 content in injection 2. Fifty-five dynamic emission images were obtained, starting at the time of the first injection, over 75 min (Fig. 1D).

Calculation of Beta-Adrenoceptor Density. Images were analyzed on SUN workstations with Analyze image analysis (*16*) and Matlab (The MathWorks, Inc., Natick, MA) mathematical software package. Regions of interest (ROIs) for the lung were drawn on the transmission images. Lung tissue on the tumor side only was analyzed. Tumor lesions, identified as high density areas in the transmission images, were excluded from normal lung regions. In the 15 planes scanned, the most caudal plane was selected from the second plane above the diaphragm. To generate pulmonary tissue tracer time-activity curves, ROIs were projected onto the dynamic (S)-[^{11}C]CGP-12177 images. The mean tracer activity in serial lung planes (cranio-caudal) was calculated and plotted against time. 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 regional (S)-[^{11}C]CGP-12177 time-activity curve. A graphical approach derived from Delforge et al. (*13*) was used to calculate the B_{max} of pulmonary beta-adrenoceptors in each ROI, taking into account the total amount of cold ligand in both injections. This technique was further modified to express B_{max} as pmole g^{-1} (pmole per gram of tissue) by normalizing B_{max} to local values of extravascular tissue density.

Theory of the Graphical Method. This approach relies on a difference in the kinetic behavior of the radioactive ligand when injected under conditions of low and high specific activity, and is based on the following differential equation:

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

where B(t) and F(t) are the molar concentrations of the bound and free ligands, 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 calculate the density of available receptors (B_{max}) in terms of measurable quantities (*13*).

The method is essentially 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.

In Vitro Measurement of Beta-Receptors

Radioligand binding assays of normal lung tissue obtained from thoracotomy and lobectomy performed one day after the preoperative PET scans were undertaken using (S)-[^{3}H]CGP-12177.

Preparation of Cell Membranes. All lung tissues obtained were from lobectomy specimens. A piece of peripheral lung (approximately 5 g wet weight) was removed for ligand binding from a region free of gross tumor. Each piece was sampled as far away from the tumor lesion as possible. An adjacent portion of the tissue was inflated with a 1:4 mixture of OCT Embedding Matrix

(CellPath, England) and phosphate-buffered saline. A suitable block was snap frozen in melting isopentane. A cryostat microtome section from this block was stained with hemotoxylin and eosin and examined under a microscope to confirm the absence of tumor and tissue apparent abnormalities. The tissue was minced, suspended in an ice-cold homogenate buffer containing 20 m*M* tris-HCl, 0.9% NaCl at pH 7.4 and then homogenized with an Ultra Turrax homogenizer (TP18-10, Janke and Kunkel KG, Staufen, Germany) at half and full settings for 1 min each. The homogenate was centrifuged in a Sorvall RC 5B centrifuge at 500 × g for 10 min at 4°C to remove the undisrupted elements and fibrous tissue. The supernatant was recentrifuged at 48,000 × g for 15 min at 4°C. The pellet was subsequently washed, resuspended and stored at −80°C until use.

The protein concentration was determined according to the methods of Lowry et al. using bovine serum albumin as the standard (*17*).

Radioligand Binding Assays. The frozen membrane preparation was allowed to thaw at 4°C and finally resuspended to a concentration of 0.5–1.0 mg ml^{-1} protein in an incubation buffer containing 20 m*M* tris-HCl, 10 m*M* $MgCl_2$, 0.9% NaCl at pH 7.4. 100 μl of membrane suspension was incubated with seven concentrations (0.06–3.2 n*M*) of (S)-[^{3}H]CGP-12177 (53 Ci/mmole, Amersham International Plc., England) at 37°C for 60 min in a total volume of 250 μl. These conditions allowed complete equilibration of the receptor with the radioligand. The reaction was stopped by adding 2 ml of ice-cold washing buffer containing 10 m*M* tris-HCl, 2 m*M* $MgCl_2$, 0.9% NaCl at pH 7.4 and immediate filtration through Whatman GF/C glassfiber filters using a Brandel cell harvester (Brandel, Biomedical Research and Development Laboratories, U.S.A.). Each filter was washed three times with 5 ml of ice-cold washing buffer to separate bound from free ligand. Filters with retained radioactivity were left overnight in 10 ml of scintillant and counted using a liquid scintillation counter. Measurements were carried out in triplicate at each concentration of (S)-[^{3}H]CGP-12177 for each experiment.

Analysis of In Vitro Saturation Binding Isotherm. Saturation curves were analyzed according to the equation:

$$B_{tot} = B_{max} \cdot F/(K_D + F) + K_{ns} \cdot F,$$

where B_{tot} (fmole mg^{-1} protein) is the observed bound (S)-[^{3}H]CGP-12177 and F (n*M*) is the free concentration of (S)-[^{3}H]CGP-12177 (original total concentration with correction for observed bound fraction). Estimates of the parameters B_{max} (fmole mg^{-1} protein), K_D (n*M*) and K_{ns} (nonspecific binding constant; μl mg^{-1} protein) were obtained with the binding computer program in the MRC Cyclotron Unit.

Scatchard plots were initially employed to analyze a preliminary study on rat lung tissue and the data from Patient 1 to estimate the binding parameters. In these cases, lung tissue membranes were incubated at each concentration of (S)-[^{3}H]CGP-12177 both with and without 80 μ*M* of (−)-isoproterenol to determine nonspecific binding experimentally. Scatchard analysis gave the results: B_{max} = 434 fmole mg^{-1} protein, and K_D = 0.136 n*M* for rat lung tissue; and B_{max} = 117 fmole mg^{-1} protein, and K_D = 0.070 n*M* for lung tissue from Patient 1. These values were then used as the starting values for the iterative computerized nonlinear curve fitting to give the final results: 463 fmole mg^{-1} protein, K_D = 0.148 n*M* for rat lung tissue; and B_{max} = 115 fmole mg^{-1} protein, K_D = 0.073 n*M* in Patient 1.

Competitive Binding Experiments. Competitive binding between (−)-propranolol, a nonselective beta-antagonist (Sigma, England), ICI 118551, a selective β_2-antagonist (Cambridge Research Biochemicals, England) or CGP 20712A, a selective β_1-antagonist (Ciba-Geigy LTD, Switzerland), and the radioligand (S)-[^{3}H]CGP-12177 for human lung membranes was performed by incubating lung membranes from Patient 2 with 0.5 n*M* of (S)-[^{3}H]CGP-12177 and a series of concentrations of the three antagonists. The competition curves were analyzed with the EBDA and LIGAND nonlinear least-square curve fitting programs (*18*).

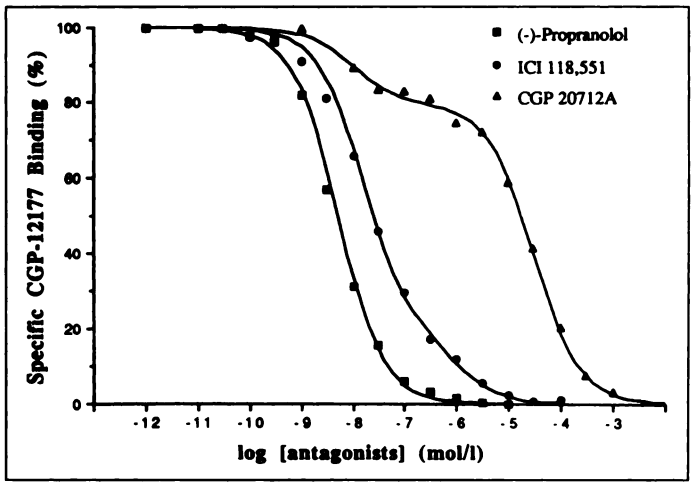

FIGURE 2. Competition for specific (S)-[^{3}H]CGP-12177 binding to human lung membranes by beta-adrenergic antagonists. Lung membranes were incubated with 0.5 n*M* (S)-[^{3}H]CGP-12177 in the presence of increasing concentration of nonselective (−)-propranolol, β_2-selective ICI 118, 551 and β_1-selective CGP 20712A.

Statistical Analysis

Data presented are mean ± s.d., unless otherwise stated. For the linear regression analysis, a $p < 0.05$ was considered statistically significant.

RESULTS

Competitive Binding In Vitro

The results of the competition experiments for Patient 2 are shown in Figure 2. This figure demonstrates that the three competitors (beta-antagonists) bind more to the receptors as their concentrations increase, thus allowing less of the radioligand (S)-[^{3}H]CGP-12177 to bind. The curve for propranolol was monophasic, while the curves of ICI 118551 and CGP 20712A were biphasic. Analysis with the ligand program generated dissociation constants (K_i) for the three competitors as summarized in Table 1.

Beta-Receptor Density, K_D and Nonspecific Binding

A representative saturation curve obtained under equilibrium conditions in vitro is shown in Figure 3. All binding parameters were estimated with the computer-assisted nonlinear curve fitting program. The results of the in vitro radioligand binding measurements and the in vivo PET measurements are summarized in Table 2. K_D in vitro was a rather variable parameter, ranging from 0.07 n*M* to 0.17 n*M* (mean 0.14 n*M*). The nonspecific binding for the group at K_D equivalent (at which (S)-[^{3}H]CGP-12177 concentration equates to K_D value) was only 3% of the total binding. In the radioligand binding study, B_{max} was 99.0 ± 15.5 fmole mg^{-1} protein. In the PET study, V_B was 0.096 ± 0.017 ml ml^{-1}, D_{ev} was 0.176 ± 0.017 g ml^{-1} and B_{max} was 9.43 ± 1.32 pmole g^{-1} tissue. A relationship was observed between beta-receptor density quantified using PET and receptor density determined by radioligand binding assay. Regression of in vivo estimates against in vitro measurements showed a significant correlation ($r = 0.92$, $p < 0.05$) (Fig. 4). In an attempt to assess the extent of agreement, a Bland-Altman plot was constructed. B_{max} obtained using PET was converted to fmole mg^{-1} protein [the percentage of tissue protein was

TABLE 1
Dissociation Constants for Beta-adrenergic Antagonists Displacing (S)-[^{3}H]CGP-12177 Binding to Human Lung Membranes

Beta-antagonists	K_i* (*M*)	$K_{i(H)}$† (*M*)	$K_{i(L)}$‡ (*M*)	β_1-subtype (%)	β_2-subtype (%)
(−)-Propranolol	$1.0 \times 10^{-9} \pm 0.1$				
ICI 118, 551		$3.0 \pm 0.5 \times 10^{-9}$	$1.9 \pm 0.5 \times 10^{-7}$	19.2	80.8
CGP 20712A		$2.1 \pm 1.3 \times 10^{-9}$	$6.1 \pm 0.4 \times 10^{-6}$	20.6	79.4

Values shown are means ± s.e.m.
* = dissociation constant of the whole beta-receptor population for the competitor, † = dissociation constant of the high affinity sites for the selective beta-antagonists, ‡ = dissociation constant of the low affinity sites for the selective beta-antagonists.

taken as 10% (*13*)] and the difference between the two methods plotted against their mean (*19*) (Fig. 5). The mean difference was found to be 4.66 fmole mg^{-1} protein. The s.d. of the differences between the two methods was 6.26 fmole mg^{-1} protein. The upper limit of the 95% confidence interval (+ 2 s.d.) for the bias was 17.2 fmole mg^{-1} protein and the lower limit was −7.9 fmole mg^{-1} protein. Thus, the PET measurement may differ from the RLBA measurement by 17 fmole mg^{-1} below and 8 fmole mg^{-1} above.

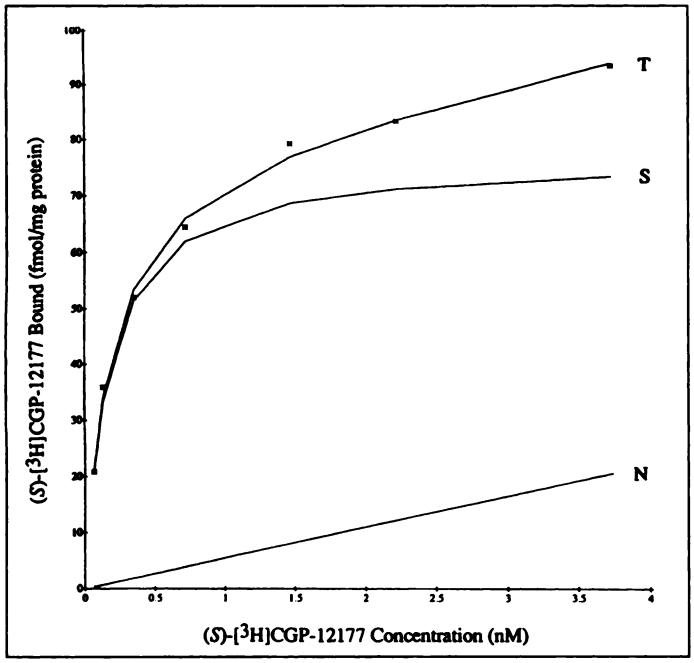

FIGURE 3. Saturation curve of (S)-[^{3}H]CGP-12177 binding to membranes of human lung tissue. Total binding (T) was determined over a broad concentration range and decomposed into specific (S) and nonspecific binding (N) with a computer-assisted nonlinear curve fitting program called binding.

TABLE 2
Summary of Binding Parameters In Vitro and In Vivo

Patient no.	K_D (*nM*)	Nonspecific (%)	B_{max} in vitro (fmole/mg prot)	B_{max} in vivo (pmole/g tissue)
1	0.073	1.7	115.0	10.62
2	0.125	3.3	89.9	7.84
3	0.171	2.0	76.6	8.16
4	0.160	6.0	107.7	10.39
5	0.164	3.7	105.8	10.16
Group	0.14 ± 0.04	3.3 ± 1.7	99.0 ± 15.5	9.43 ± 1.32

Values shown for the group are means ± s.d. K_D = equilibrium dissociation constant; Nonspecific = nonspecific binding/total binding at K_D equivalent; B_{max} in vitro = receptor density estimated by radioligand binding assay; B_{max} in vivo = receptor density estimated by PET; fmole/mg prot = fmole/mg protein.

DISCUSSION

This study was performed to assess in vivo quantification of pulmonary beta-receptors in humans using (S)-[^{11}C]CGP-12177 and PET. CGP-12177 has been shown to satisfy the criteria required for specific binding of a ligand to its receptor

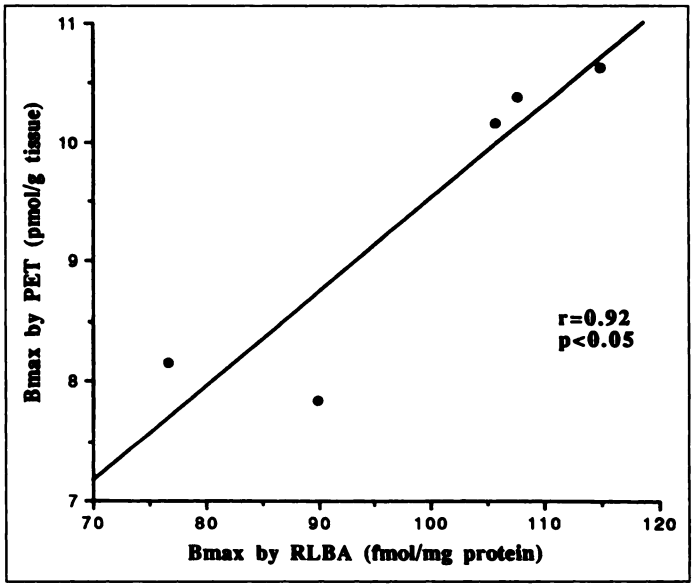

FIGURE 4. Correlation between pulmonary beta-adrenoceptor density obtained by PET and by radioligand binding assay.

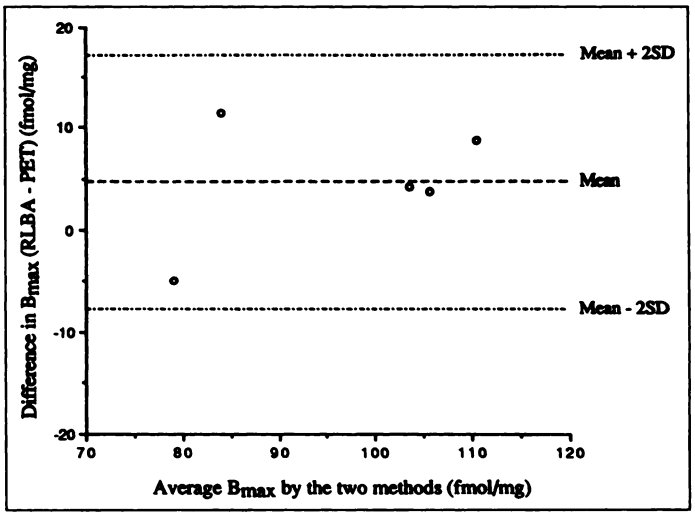

FIGURE 5. Bland-Altman plot of the PET and RLBA measurements of B_{max}.

both in vitro and in vivo: high affinity, saturability, specificity and sterospecificity (*20–22*).

Competition Studies. Propranolol is one of the most commonly used nonselective beta-antagonists, while ICI 118551 and CGP 20712A are relatively new selective beta-antagonists. The previously reported values obtained with (−)-[^{125}I]-iodocyanopindolol (*23*) for the binding affinity of ICI 118551 with β_1 and β_2 adrenergic receptors are 0.13 μM and 0.72 nM, respectively. The corresponding values for CGP 20712A are 0.9 nM and 6.3 μM. The large difference between their K_i values for β_1 and β_2 subtypes renders them the best ligands so far to study the subpopulations of beta-adrenoceptors. Since the study of beta-adrenoceptors in human lungs using (S)-[^{3}H]CGP-12177 as radioligand has not been reported, it is necessary to show specific (S)-[^{3}H]CGP-12177 binding to beta-adrenoceptors. This was achieved in the present study by demonstrating competition between (S)-[^{3}H]CGP-12177 binding to human lung membranes and the beta-antagonists mentioned above. Furthermore, the current study demonstrated that the three antagonists competed for (S)-[^{3}H]CGP-12177 with the appropriate potency. Our results (Table 1) are in agreement with the typical values obtained previously using (S)-[^{125}I]-iodocyanopindolol (*23*) and are consistent with the finding that the majority of beta-receptors found in the human lung are of the β_2 subtype (*1*).

Analysis of In Vitro Saturation Curves for (S)-[^{11}C]CGP-12177. There are two approaches to analyzing the results of radioligand binding experiments: Scatchard plot analysis and nonlinear regression analysis. We chose the latter for several reasons. First, nonlinear regression allows one to fit experimental data directly, in terms of specific and nonspecific binding, and avoids a linear transformation (e.g., Scatchard plot), which can magnify experimental error. This provides a powerful means of analyzing radioligand binding data (*24*). Secondly, in order to perform Scatchard plot analysis, nonspecific binding needs to be obtained by the incubation of tissue samples in the presence of an unlabeled drug that binds to virtually all the receptors. Hence, any inappropriate concentration of this drug or inappropriate choice of the blocking drug itself will lead to an over or underestimation of the receptor density. Nonlinear regression analysis on the other hand, avoids the need to make experimental determinations of nonspecific binding altogether, relying instead on a mathematical definition. In the current study, nonlinear regression was performed on the data for total binding using an equation describing one saturable (specific) and one nonsaturable (nonspecific) class of binding sites.

Equilibrium Dissociation Constant (K_D). (S)-CGP-12177 is generally considered to be a nonselective beta-antagonist (with less than twofold selectively towards the β_1 subtype) (*25*). The K_D of 0.14 nM obtained in the current in vitro binding study on human lung tissue was close to the previously reported values: 0.11 nM (*26*) and 0.20 nM (*20*) in glioma cells, 0.29 nM (*27*) in guinea-pig lung tissue and 0.40 nM (*25*) in rat myocardial membranes. These values indicate that (S)-CGP-12177 is a beta-receptor ligand with high affinity. It is more potent than dihydroalprenolol (DHA) which has a K_D value of 0.5 nM (*20*), a commonly used tritiated beta-antagonist ligand. An in vivo K_D, however, cannot be obtained using the present approach with PET. Calculation of K_D needs values for the parameters k_{+1}, V_R and k_{-1} (see MATERIALS AND METHODS for the definition of the terms), none of which are known. Measurement of k_{-1} would require administration of a saturating dose of a beta-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 using pindolol (*28*), large doses (>30 mg intravenously) would be required in humans, which is not a practical proposition. Thus, K_D was not determined in vivo.

Nonspecific Binding. The short life of ^{11}C ($T_{1/2}$ = 20.4 min) and the fact that a rapid intravenous injection of a large dose of propranolol is impractical rules out the in vivo measurement of the nonspecific binding fraction in humans by means of a displacement experiment. In a previous study on rat lung (*21*), nonspecific binding in vivo was shown to be ~6% of the total binding, as determined by tissue-to-plasma ratios of 9.6 and 170 with and without a blocking dose of propranolol (7 μmole kg^{-1} intravenous bolus). In a similar study in humans, a 20-min infusion of 20 mg propranolol was administered prior to injecting (S)-[^{11}C]CGP-12177 to block specific binding. This resulted in a lower lung tissue-to-plasma ratio of 6.2, compared to 108 without blocking (*29*). These findings suggest that (S)-CGP-12177 binds to beta-receptors in lung in vivo with less than 6% nonspecific binding. Moreover, nonspecific binding determined in the present in vitro experiment varied between 2% and 6%, which agrees well with previous in vitro studies showing nonspecific binding being <5% (*20,26*). These data confirm the impressively low nonspecific binding value of (S)-CGP-12177. The quantification of pulmonary beta-receptor density using (S)-[^{11}C]CGP-12177 is greatly simplified if the measurement of nonspecific binding (~6% in humans) can be omitted. This is important since beta-blocker administration would be a contraindication in some clinical cases (e.g., asthma) and since performing paired PET scans to determine nonspecific binding in every subject would be costly and would expose subjects to an undue amount of radiation.

Functional Characteristics of (S)-[^{11}C]CGP-12177. One of the characteristics of (S)-[^{11}C]CGP-12177 is that it is metabolized at an extremely slow rate in humans. Metabolite studies (*30*) have shown that more than 97% of the plasma radioactivity was unchanged (S)-[^{11}C]CGP-12177 one hour after intravenous injection. This was important, since PET measures ^{11}C indiscriminately.

A further merit is that CGP-12177 is hydrophilic, unlike other radioligands ([e.g., [^{3}H]-DHA, [^{125}I]-iodocyanopindolol and [^{3}H]-carazolol]) which are lipophilic. Because of its high hydrophilicity, (S)-[^{11}C]CGP-12177 binds only to surface membrane receptors, the majority of which are functionally coupled to adenylyl cyclase. Thus, (S)-[^{11}C]CGP-12177 does not bind to internalized receptors and will not be trapped inside cells. Therefore, the signal detected is functionally relevant and nonspecific binding is very low. In theory, (S)-[^{11}C]CGP-12177 could detect alterations in the membranes of cell surface receptors that occurred without any change in the total number of receptors.

In Vivo-In Vitro Comparison. In the radioligand binding assay, the mean B_{max} for the five resected lung tissue samples was 99.0 ± 15.5 fmole mg^{-1} protein. In the PET study, the mean B_{max} was 9.43 ± 1.32 pmole g^{-1} tissue. Using [^{125}I]-cyanopindolol, Carstairs et al. (*1*) studied the binding characteristics of tissue sections and membranes prepared from human lung. B_{max} was 126 ± 8.6 fmole mg^{-1} protein (mean ± s.e.m., n = 9) for the sections and 95 ± 5.5 fmole mg^{-1} protein (mean ± s.e.m., n = 3) for the membrane preparations. Using another commonly used ligand, [^{125}I]-pindolol, Liggett et al. (*31*) obtained a similar B_{max} value of 83 ± 10 fmole mg^{-1} protein (mean ± s.e.m., n = 11) for human lung membranes. All the above values are in close agreement with the current results. These values, however, are lower than those reported by Hauck et al. (*8*), who obtained receptor densities of 235 ± 26 fmole mg^{-1} protein using human lung membrane homogenates.

The in vivo value of B_{max} reported in this paper (9.43 ± 1.32 pmole g^{-1}) is lower than our previously determined value of 14.8 ± 1.6 pmole g^{-1} for normal lung tissue (*3*). This difference may arise for two reasons. First, the extravascular density (D_{ev}) value determined for our group of patients (0.176 g ml^{-1}) and for groups of normals currently being studied (0.16 to 0.20 g ml^{-1}) is higher than that reported by Ueki et al. (0.137 g ml^{-1}) (*3*). Because of the consistently higher range of values currently obtained, we feel that the lower D_{ev} value obtained by Ueki et al. may have been incorrectly low, resulting in falsely high B_{max} values when expressed per gram of lung tissue. Had a D_{ev} value of 0.137 g ml^{-1} been found in this study, however, it would have resulted in a B_{max} value of only 12.1 pmole g^{-1}. This value is still lower than that found by Ueki et al. and suggests that the receptor density of lung tissue in these tumor patients is less than that found in normals. This may arise because of an age difference between the patients studied here (57 ± 9 yr) and the normals studied previously (26 yr: range 21–34 yr), an effect of stress on the patients prior to the thoracotomy (resulting in a possible down-regulation of receptor density) or the effect of active agents on the receptors produced by the tumors themselves.

More important, however, was the portion of the present study that showed that the beta-receptor density estimates in vivo correlated with those obtained in vitro, using the same ligand and the same lung for the comparison. Thus, compared with the classic method of radioligand binding assay, PET appears to be a sensitive and accurate means of quantifying human pulmonary beta-receptors in vivo. B_{max} values obtained using PET have been normalized by extravascular lung tissue density to provide the value of receptor density with units of pmole g^{-1} tissue, whereas B_{max} obtained in vitro was normalized to tissue protein content to provide units of fmole mg^{-1} protein. If the tissue protein content of wet tissue is taken to be 10% (*13*), the results of the two measurements are similar in absolute terms. In view of the different physiological milieu prevailing at the time of the in vivo and in vitro measurements and the different normalization techniques used for the two methods (per g extravascular tissue for PET and per mg protein for in vitro), the similarity of these two values is somewhat fortuitous. Even so, the correlation between the PET and radioligand binding methods (Fig. 4) suggests that they may broadly be measuring the same receptor populations.

Subpopulations of the Beta-Receptor Pool. PET measurements quantify the total pulmonary beta-receptor pool, which is dominated by beta-receptors on the alveolar wall. In view of their role in modulating bronchial tone, beta-receptors in bronchial smooth muscle are obviously important, although beta-receptors in the bronchial epithelium have also been reported to be relevant (*32*). It appears unlikely, however, that receptor subpopulations would change in opposite directions after therapeutic interventions with beta-agonists, although the baseline level (*1*) and the extent of the change in each subpopulation may differ. We have recently studied a group of seven normal subjects (*33*) in which PET measurements of beta-receptor density and bronchodilator response to salbutamol (a β_2-agonist) were performed before and after 2 wk of salbutamol therapy. Following chronic β_2-agonist dosing, a significant reduction in receptor density was observed. This was associated with a mild tachyphyllaxis in bronchodilator response, suggesting that the total pulmonary beta-receptor pool and receptors in airway smooth muscle behave similarly after doses of β_2-agonists.

Safety of the Method. In addition to the cancer patients investigated in the present study, we also scanned 29 normal subjects. No subjects have experienced problems associated with the CGP-12177 injection. None of these subjects became dyspneic. FEV_1 and FVC were recorded in earlier studies (n = 7), but there was no significant change in them. No changes in blood pressure and electrocardiogram were observed either. With a similar approach, Merlet et al. (*34*) studied cardiac beta-receptors in ten patients with heart failure related to idiopathic cardiomyopathy, as well as in eight normal subjects. They observed no change in blood pressure but a 16% reduction in heart rate. Two patients showed transient and minor dyspnea. As in our study, however, they did not see any significant changes in heart rate or blood pressure in normal controls. We have also studied nine mild to moderate asthmatics, for whom a CGP-12177 challenge test (3 μg followed by 25 μg cold CGP-12177) was given intravenously several days before the PET scan. A 20% reduction in FEV_1 or FVC was considered to show hyper-reactivity to the ligand with exclusion of the subject from the study. This challenge induced bronchospasm in only one patient who did not go on to have the PET scan. His symptoms were relieved after inhalation of salbutamol. We believe this PET technique is well tolerated by normal subjects, but suggest that a cold CGP-12177 challenge test be given to asthmatic patients and any subjects with airway hyper-reactivity prior to the PET scan.

CONCLUSION

This study demonstrates the efficacy of a new in vivo approach to investigate pulmonary beta-adrenoceptors in humans using PET and (S)-[^{11}C]CGP-12177. This new technique was compared with a classic method (radioligand binding assay) that is ordinarily used to measure receptor density. The pulmonary beta-receptor density of patients who were to undergo thoracotomy, due to lung cancer, was first determined in vivo using PET and, within 24 hr, in vitro using radioligand binding assay. The in vivo results correlated well with those obtained in vitro. By comparison, PET appears to be a reliable means to quantify beta-adrenergic receptors in vivo in the human lung. The technique involves two injections of ligand (<30 μg CGP-12177) and has been shown to be well tolerated in normal subjects. It is noninvasive and can be repeated after therapeutic interventions, thereby making it possible to study the physiological and pharmacological regulation of beta-receptors in both healthy and diseased lungs.

ACKNOWLEDGMENTS

We thank S. Van Noorden in the Department of Histopathology for assistance in processing the lung specimens and Professor J. MacDermot in the Department of Clinical Pharmacology for technical advice. We also thank A. Suter of Ciba-Geigy (Switzerland) for the gift of CGP 20712A and Dr. L-H Johansson (Lund, Sweden) for his helpful communications. This work was supported by the National Asthma Campaign, Allen and Hanbury Ltd. and the British Lung Foundation.

REFERENCES

1. Carstairs JR, Nimmo AJ, Barnes PJ. Autoradiographic visualization of beta-adrenoceptor subtypes in human lung. *Am Rev Respir Dis* 1985;132:541–547.
2. Davis PB. Autonomous function in patients with airway obstruction. In: Kaliner MA, Barns PJ, eds. *The airways. Neural control in health and disease.* New York: Marcel Dekker;1988:87–118.
3. Ueki J, Rhodes CG, Hughes JMB, et al. In vivo quantification of pulmonary beta-adrenoceptor density in humans with (S)-[^{11}C]CGP-12177 and PET. *J Appl Physiol* 1993;75:559–565.
4. Brodde O-E, Brinkman M, Schemuth R, O'Hara N, Daul A. Terbutalin-induced desensitization of human lymphocyte β_2-adrenoceptors: accelerated restoration of beta-adrenoceptor responsiveness by prednisone and ketotifen. *J Clin Invest* 1985;76:1096–1101.
5. Brooks SM, McGowan K, Bernstein IL, Altenau P, Peagler J. Relationship between

numbers of beta-adrenergic receptors in lymphocytes and disease severity in asthma. *J Allergy Clin Immunol* 1979;63:401–406.
6. Spina D, Rigby PJ, Paterson JW, Goldie RG. Autoradiographic localization of beta-adrenoceptors in asthmatic human lung. *Am Rev Respir Dis* 1989;140:1410–1415.
7. Hamid QA, Mak JCW, Sheppard MN, et al. Localization of β_2-adrenoceptor messenger RNA in human and rat lung using in situ hybridization: correlation with receptor autoradiography. *Eur J Pharmaco (Molec Pharmacol)* 1991;206:133–138.
8. Hauck RW, Bohm M, Gengenbach S, et al. β_2-adrenoceptors in human lung and peripheral mononuclear leukocytes of untreated and terbutaline-treated patients. *Chest* 1990;98:376–381.
9. Bohm M, Gengenbach S, Hauck RW, Sunder PL, Erdmann E. Beta-adrenergic receptors and m-cholinergic receptors in human lung. Findings following in vivo and in vitro exposure to the beta-adrenergic receptor agonist, terbutaline. *Chest* 1991;100:1246–1253.
10. Tashkin DP, Conolly MC, Deutsch RI, et al. Subsensitization of beta-adrenoceptors in airways and lymphocytes of healthy and asthmatic subjects. *Am Rev Respir Dis* 1982;125:185–193.
11. Homcy CJ, Stauss HH, Kopiwoda S. Beta-receptor occupancy: assessment in the intact animal. *J Clin Invest* 1980;65:1111–1118.
12. Hughes B, Marshall DR, Sobel BE, Bergmann SR. Characterization of beta-adrenoceptors in vivo with iodine-131 pindolol and gamma scintigraphy. *J Nucl Med* 1986;27:660–667.
13. Delforge J, Syrota A, Lancon J-P, et al. Cardiac beta-adrenergic receptor density measured in vivo using PET, CGP 12177 and a new graphical method. *J Nucl Med* 1991;32:739–748.
14. Brady F, Luthra SK, Tochon-Danguy H-J, et al. Asymmetric synthesis of a precursor for the automated radiosynthesis of S-(3′-t-butylamino-2′-hydroxypropoxy)-benzimidazol-2-[^{11}C] one (S-[^{11}C]CGP-12177 as a preferred radioligand for beta-adrenergic receptors. *Appl Radiat Isot* 1991;42:621–628.
15. Rhodes CG, Wollmer P, Fazio F, Jones T. Quantitative measurement of regional extravascular lung density using positron emission and transmission tomography. *J Comput Assist Tomogr* 1981;5:783–791.
16. Robb RA, Hanson DP. A software system for interactive and quantitative visualization of multidimensional biomedical images. *Australas Phys Eng Sci Med* 1991;14:9–30.
17. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:265–275.
18. Macpherson GA. Analysis of radioligand binding experiments: a collection of computer programs for the IBM PC. *J Pharmacol Methods* 1985;14:213–228.
19. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986:307–310.
20. Staehelin M, Hertel C. [^{3}H]CGP-12177, a hydrophilic beta-adrenergic ligand reveals high affinity binding of agonists to intact cell. *J Biol Chem* 1983;258:3496–3502.
21. Law MP. Demonstration of the suitability of CGP 12177 for in vivo studies of beta-adrenoceptors. *Brit J Pharmacol* 1993;109:1101–1109.
22. Waarde AV, Meeder JG, Blanksma PK, et al. Uptake of radioligands by rat heart and lung in vivo: CGP 12177 does and CGP 20505 does not reflect binding to beta-adrenoceptors. *Eur J Pharmacol* 1992;222:107–112.
23. Summers RJ. Localization and regulation of beta-adrenoceptor subtypes. In: Wharton J, Polak J, eds. *Receptor autoradiography*. Oxford: Oxford University Press;1993:297–315.
24. Motulsky HJ, Insel PA. In vitro methods for studying human adrenergic receptors: methods and applications. In: Insel PA, ed. *Adrenergic receptors in man*. New York: Marcel Dekker;1987:139–160.
25. Tsuchihashi H, Yokoyama H, Nagatomo T. Binding characteristics of ^{3}H-CGP 12177 to beta-adrenoceptors in rat myocardial membranes. *Jpn J Pharmacol* 1989;49:11–19.
26. Affolter H, Hertel C, Jaeggi K, Portenier M, Staehelin M. (−)-S-[^{3}H]CGP-12177 and its use to determine the rate constants of unlabeled beta-adrenergic antagonists. *Proc Natl Acad Sci* 1985;82:925–929.
27. Johansson L-H, Persson H, Rosengren E. The role of Mg^{2+} on the formation of the ternary complex between agonist, beta-adrenoceptor and Gs-protein and an interpretation of high and low affinity binding of beta-adrenoceptor agonists. *Pharmacol Toxicol* 1992;70:192–197.
28. Rhodes CG, Hughes JMB, Araujo LI, et al. Pulmonary beta-receptor imaging in vivo using PET and active enantiomer of CGP 12177-labeled with ^{11}C [Abstract]. *Thorax* 1991;46:757P.
29. Rhodes CG, Hughes JMB. Pulmonary studies using PET. *Eur Respir J* 1995;8:1001–1017.
30. Luthra SK, Osman S, Steel CJ, et al. Comparison of S-[^{11}C] CGP 12177 metabolism in rat, dog and man using solid phase extraction and HPLC. *J Labd Compd Radiopharm* 1993;32:504–505.
31. Liggett SB, Marker JC, Shah SD, Roper CL, Cryer PE. Direct relationship between mononuclear leukocyte and lung beta-adrenergic receptors and apparent reciprocal regulation of extravascular, but not intravascular, alpha- and beta-adrenergic receptors by the sympathochromaffin system in humans. *J Clin Invest* 1988;82:48–56.
32. Morrison KJ, Gao Y, Vanhoutte PM. Beta-adrenoceptors and the epithelial layer in airways. *Life Sci* 1993;52:2123–2130.
33. Hayes M, Qing F, Rhodes CG, et al. Effects of two weeks of salbutamol on human pulmonary beta-adrenergic receptor number and airway function. *Eur Respir J* 1994;7(suppl 18):235S.
34. Merlet P, Delforge J, Syrota A, et al. PET with ^{11}C-CGP-12177 to assess beta-adrenergic receptor concentration in idiopathic dilated cardiomyopathy. *Circulation* 1993;87:1169–1178.

Overall Accuracy of Technetium-99m-MAG3 Clearance Measurements Obtained with a Gamma Camera Heart Curve

Marianne Tondeur, Jacques Kinthaert, Amnon Piepsz and Hamphrey R. Ham
Department of Nuclear Medicine, Saint-Pierre Hospital, Free Universities of Brussels, Brussels, Belgium

The aim of the present study was to evaluate the accuracy of ^{99m}Tc-MAG3 clearance measurements using a precordial gamma camera curve calibrated by a single plasma sample. **Methods:** Technetium-99m-MAG3 was administered to ten young normal volunteers. A 60-min gamma camera acquisition was performed. Five different segments of the gamma camera curve were determined: 3 min to 20 min, 3 min to 30 min, 3 min to 40 min, 3 min to 50 min and 3 min to 60 min. A biexponential function was fitted on each of these five different segments, which were thereafter calibrated using eight different blood samples. These blood samples were successively used for calibration at 5, 10, 15, 20, 30, 40, 50 and 60 min. The single injection, multiple plasma sample method was used as reference. **Results:** Camera clearances varied markedly based on the length of the precordial curve and on the time of the calibration sample. Different regression equations were obtained for each duration of the camera curve, and for each blood sample timing. Correlation coefficients were >0.95 in most cases recording period of at least 50 min, however, was necessary to obtain a s.e.e. better than those obtained using a single blood sample method without gamma camera curve. **Conclusion:** The ^{99m}Tc-MAG3 clearance determination using a gamma camera heart curve calibrated with a single blood sample does not necessarily improve the accuracy of the one blood sample method.

Key Words: technetium-99m-MAG3; gamma camera; renal clearance

J Nucl Med 1996; 37:1281–1284

Reference methods that allow calculation of the global renal clearance include the continuous infusion method and the single injection, multiple plasma sample method. In clinical practice, simplified methods using one or fewer blood samples are more often used (*1–7*). Reduction in the number of blood samples, however, often accounts for a reduced accuracy; for example,

Received May 23, 1995; revision accepted Dec. 31, 1995.
For correspondence or reprints contact: Marianne Tondeur, MD, Department of Nuclear Medicine, University Hospital Saint-Pierre, 322, Rue Haute, B-1000 Brussels, Belgium.

The Journal of NUCLEAR MEDICINE

In Vivo Quantification of Human Pulmonary Beta-Adrenoceptor Density Using PET: Comparison with In Vitro Radioligand Binding

Feng Qing, Christopher G. Rhodes, Michael J. Hayes, Thomas Krausz, S. William Fountain, Terry Jones and J.M.B. Hughes

J Nucl Med. 1996;37:1275-1281.

The Journal of Nuclear Medicine is published monthly.
SNMMI | Society of Nuclear Medicine and Molecular Imaging
1850 Samuel Morse Drive, Reston, VA 20190.
(Print ISSN: 0161-5505, Online ISSN: 2159-662X)

SNMMI SOCIETY OF NUCLEAR MEDICINE AND MOLECULAR IMAGING