In Vivo Quantification of Human Pulmonary β-Adrenoceptors: Effect of β-Agonist Therapy

MICHAEL J. HAYES, FENG QING, CHRISTOPHER G. RHODES, SHAKIL U. RAHMAN, PHILIP W. IND, SHIRANEE SRISKANDAN, TERRY JONES, and J. M. B. HUGHES

Medical Research Council, Clinical Sciences Center, Cyclotron Unit and Department of Medicine (Respiratory Division), Royal Postgraduate Medical School, Hammersmith Hospital, London, United Kingdom

In human subjects, chronic β_2-agonist dosing reduces mononuclear leukocyte (MNL) β-adrenoceptor numbers. The aim of this study was to investigate whether this downregulation also occurs in the lung. Seven healthy male subjects were treated for 2 wk with oral (up to 16 mg/d) and inhaled (up to 1.6 mg/d) albuterol (salbutamol in Europe). Pulmonary maximal β-adrenoceptor binding capacity (Bmax) was determined *in vivo* using positron emission tomography (PET) and the β-receptor antagonist ligand, ^{11}C-labeled CGP-12177, before and after the 2-wk chronic dosing. MNL Bmax was also measured, using a radioligand binding assay and ^{3}H-labeled CGP-12177. Bronchodilator responses to the β_2-agonist were determined after each PET scan by measuring the change in specific airway conductance (SGaw) after increasing doses of inhaled albuterol. Pulmonary and MNL Bmax fell by 22% ± 14% ($p < 0.05$) and 42% ± 19% ($p < 0.05$) respectively. The changes in pulmonary and MNL Bmax were correlated ($r = 0.9$, $p < 0.05$). There was also a reduction in the bronchodilator response to inhaled albuterol. In a further six subjects, pulmonary and MNL Bmax did not change during an acute infusion of albuterol (2 to 4 μg/kg/h). The reduction in pulmonary β-adrenoceptor numbers after chronic albuterol dosing may be predictable from the changes observed in circulating MNL cells. Hayes MJ, Qing F, Rhodes CG, Rahman SU, Ind PW, Sriskandan S, Jones T, Hughes JMB. *In vivo* quantification of human pulmonary β-adrenoceptors: effect of β-agonist therapy.

AM J RESPIR CRIT CARE MED 1996;154:1277–1283.

Inhaled β_2-agonists are the most effective and widely used bronchodilator therapy for asthma. However, since shortly after their introduction, there have been concerns about their safety. In the late 1970s there was an increase in asthma mortality in New Zealand (1). Epidemiologic studies in New Zealand (2) and subsequently in Canada (3) suggested that overuse of β_2-agonists, particularly fenoterol may have been associated with an increased risk of death from asthma. Although an association between β_2-agonists and death from asthma was demonstrated, these epidemiologic studies were unable to prove a causal relationship between the two.

Clinical trials examining the chronic effects of inhaled β_2-agonists on bronchial responsiveness and asthma control have given mixed results. Regular as opposed to "as required" fenoterol was associated with poorer asthma control (4), but this was not shown in a similar study with albuterol (5).

While epidemiologic and clinical studies have left the issue of adverse effects from chronic β_2-agonist therapy unresolved, there are many studies showing that tachyphylaxis to some of the effects of β_2-agonists does occur. This has been shown for heart rate changes (6), as well as for the bronchodilator response in normal subjects (7). More importantly, the protective effect of β_2-agonists against the bronchoconstriction induced by exercise (8), and allergen (9) in asthma also decreases with regular β_2-agonist use.

At the level of the receptor there is evidence that this tachyphylaxis is mediated by downregulation and uncoupling of β_2-adrenoceptors. The earliest change to occur in the presence of β_2-agonists seems to be a shift of the receptor from a high-affinity state to a low-affinity state (10). This is associated with a loss of adenylate cyclase activity of the receptor (11). Internalization of the receptor, also associated with loss of function, may occur at a relatively early stage (12). After further exposure to β_2-agonists, synthesis of β_2-adrenoceptors is depressed as evidenced by reduced receptor messenger RNA (mRNA) expression (13).

In human subjects this reduction in β_2-adrenoceptor number after exposure to β_2-agonists *in vivo* has been repeatedly demonstrated using circulating lymphocytes (14–16). There have been few studies on the effects of β_2-agonists given *in vivo* on pulmonary β-adrenoceptor number. Autoradiographic studies of human lung have suggested that β-adrenoceptors are widely distributed throughout the lung with more than 90% of the total pulmonary adrenoceptors residing in alveolar walls (17). Until recently, quantification of these pulmonary receptors has only been possible *in vitro*. Radioligand binding studies using *in vitro* measurements on lobectomy specimens have not shown any significant change in pulmonary β-adrenoceptor density in patients treated with β_2-agonists prior to the operation (18).

Positron emission tomography (PET) has made possible the

(Received in original form November 27, 1995 and in revised form March 11, 1996)

Supported by Allen & Hanburys Ltd., the National Asthma Campaign, and the British Lung Foundation.

Correspondence and requests for reprints should be addressed to Professor J. M. B. Hughes, Department of Medicine (Respiratory Division), Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Rd., London W12 0NN, UK.

noninvasive quantification of β-adrenoceptor density *in vivo* using the radiolabeled ligand ^{11}C-CGP12177 (19, 20). This permits the serial measurement of pulmonary β-adrenoceptor density in individual subjects. The aim of this study was to investigate: the effect of β_2-agonists on human pulmonary β-adrenoceptors; the relationship between changes in pulmonary β-adrenoceptors and the bronchodilator response to inhaled albuterol; and the relationship between changes in pulmonary β-adrenoceptors and changes in mononuclear leukocyte (MNL) β-adrenoceptors.

METHODS

Subjects

All subjects were normal volunteers who were recruited locally. Because of the radiation dose associated with PET scanning, women of childbearing age were excluded. Subjects with a history of any significant respiratory or other medical illness were also excluded. None were taking any medications at the time of the study. Subjects participated in this study after giving written and informed consent to the relevant protocols which were approved by the Ethics Committee of the Hammersmith and Queen Charlotte's Special Health Authority and by the United Kingdom Administration of Radioactive Substances Advisory Committee (ARSAC).

Study Design

Eighteen subjects were studied in the supine position after resting for 30 min using three different protocols.

Study 1. Five subjects had measurements of pulmonary and peripheral blood MNL β-adrenoceptor density on two separate occasions without any intervention to assess the reproducibility of the techniques.

Study 2. In seven subjects, pulmonary and MNL β-adrenoceptor density, as well as bronchodilator response to albuterol, were measured before, and 16 h after completion of 2 wk of regular treatment with high-dose oral and inhaled albuterol.

Study 3. In six subjects, baseline measurements of pulmonary and MNL β-adrenoceptor density were made and then repeated 1 wk later (without the chronic dosing regimen) during an acute infusion of albuterol. This study was designed to reproduce the residual levels of plasma albuterol which were present after the chronic dosing regimen of Study 2, to assess competition between plasma albuterol and ^{11}C-CGP12177 on the *in vivo* β-adrenoceptor measurement.

Treatment

In Study 2, subjects received 200 μg inhaled albuterol four times daily and slow-release albuterol (Volmax) 4 mg orally twice daily for the first week. During the second week, subjects took 400 μg inhaled albuterol four times daily and slow release albuterol 8 mg orally twice daily. Treatment was stopped 16 h before measurement of pulmonary and MNL β-adrenoceptors.

In Study 3, an infusion of albuterol was commenced 45 min before the injection of ^{11}C-CGP12177 during the second PET scan. The infusion was administered through a cubital fossa vein with the subjects supine. It was continued for a total of 2 h and stopped at the same time as the PET scan was completed. Infusion rates varied from 2 to 4 μg albuterol/kg/h to provide a spread of plasma levels comparable to the residual plasma albuterol levels recorded during the second PET scan in Study 2.

Measurement of Pulmonary β-Adrenoceptor Density

The preparation of the (*S*)-[^{11}C]CGP-12177, the PET scanning, and the calculation of β-adrenoceptor density, were performed as previously reported (20, 21). The nonselective-hydrophilic β-antagonist (*S*)-CGP-12177 was used for the β-receptor ligand in all the studies. This was labeled with the positron emitting radionuclide carbon-11 which has a half-life of 20.4 min. PET scans were performed using an ECAT 931-08/12 15-plane positron tomograph (Siemens/CTI, Knoxville, TN). The protocol comprised: transmission scan (for lung density), oxygen-15-labeled carbon monoxide (C^{15}O) emission scan (for blood volume), and (*S*)-[^{11}C]CGP-12177 dynamic emission scanning to measure β-adrenoceptor density. Regions of interest were drawn on the transmission scans as previously described (20). The binding capacity (Bmax) of pulmonary β-adrenoceptors in these regions was calculated from a graphical approach derived from Delforge and colleagues (19). This technique relies on the relationship between Bmax and the rate of uptake of ligand into the region of interest. Two injections of (*S*)-[^{11}C]CGP-12177 are given during the dynamic scan (each containing different amounts of inactive CGP-12177) and the rates of uptake used to solve this relationship for Bmax. This technique doe not provide a value for K_d (the equilibrium dissociation constant). The measurement of Bmax with this method is independent of K_d.

Regional pulmonary blood volume was measured following a 4-min inhalation of C^{15}O gas (20). Vascular density (g blood/ml thorax) was obtained by multiplying blood volume (ml blood per ml thoracic volume) by 1.06 (whole blood density in g/ml). Lung density (blood and extravascular tissue) obtained from the normalized transmission scan (22) was expressed as grams of lung per milliliter of thoracic volume (g/ml). Extravascular tissue density (g/ml) was calculated by subtracting vascular density from the lung density scan. Bmax was expressed as picomoles per gram of extravascular tissue (pmol/g).

Measurement of Mononuclear Leukocyte β-Adrenoceptor Density

MNLs were isolated using the technique described by Boyum (23). In summary, a heparinized sample of venous blood was diluted with Hanks' balanced salt solution (HBSS) (GIBCO, Paisley, UK), layered onto histopaque (Sigma, Poole, UK) and centrifuged. The MNL band was harvested, washed repeatedly, and stored at −70° C. On the day of the assay the cell were lysed and homogenized before being suspended in Tris-HCl incubation buffer containing 50 mM Tris, 10 mM MgCl, 25 mM sucrose, at pH 7.4 for binding assay.

The radioligand binding assay was performed and analyzed in a similar fashion to that previously described (21). Within each experiment, measurements were carried out in duplicate at each concentration of (*S*)-[^{3}H]CGP-12177. MNL membranes (50 to 100 μg of protein) were incubated with seven concentrations (0.06 to 3.2 nM) of (*S*)-[^{3}H]CGP-12177 (53 Ci/mmol; Amersham International, Aylesburg, UK) at 37° C for 60 min in a total volume of 500 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 mM Tris, 2 mM $MgCl_2$, 0.9% NaCl at pH 7.4 and by immediate filtration through Whatman GF/C filters using a Brandell cell harvester (Brandell; Biomedical Research and Development Laboratories, Gaithersburg, MD). Each filter was washed with 5 ml of ice-cold washing buffer three times to separate bound ligand from free. Filters with retained radioactivity were left overnight in 10 ml of scintillant (Hionic-Fluor; Packard, Pangbourne, UK) and then counted using a liquid scintillation counter (Beckman LS 6800; Cowley, Oxford, UK). Protein was determined according to the procedure of Lowry. Estimates of the binding parameters were obtained with a nonlinear least squares curve fitting program called "binding" as used on the Sun workstations in the MRC Cyclotron Unit. Maximal β-adrenoceptor binding capacity (Bmax) has been expressed as femtomoles per milligram of protein.

Bronchodilator Response

Bronchodilator response was assessed and analyzed as originally reported by Holgate and colleagues (7). Specific airway conductance (SGaw) was measured using a computerized body plethysmograph as the mean of six determinations. SGaw was measured 10 min after each inhalation step consisting of normal saline, albuterol 25 μg, 75 μg, 200 μg, and 400 μg administered through a breath-activated dosimeter (Mefar, Brescia, Italy). The change from baseline (ΔSGaw) was plotted against the cumulative dose of albuterol.

Plasma Albuterol

To check for the presence of residual albuterol, 16 h after dosing, during the second PET scan in Study 2, a plasma sample was taken immediately before injecting (*S*)-[^{11}C]CGP-12177. In the albuterol infusion study (Study 3) plasma samples were taken 15, 45, and 75 min after the injection of [^{11}C]CGP-12177, which was 60, 90, and 120 min after the start of the albuterol infusion. The average of these three samples has been reported. Albuterol was assayed using high-performance liquid chromatography (HPLC). The limit of detection for albuterol was 1 ng/ml (24).

Plasma Catecholamine Concentrations

Endogenous plasma catecholamine concentrations were determined in blood. Samples were taken 25 and 60 min after the injection of [^{11}C]CGP-12177 and kept on ice until plasma was separated. The samples were then stored at −70° C until assayed using HPLC with electrochemical detection. The limits of detection for epinephrine and norepinephrine were 0.05 ng/ml and 0.18 ng/ml respectively (25).

Missing Data

One albuterol sample, one MNL sample, and five catecholamine samples were lost when a freezer failed.

Statistical Methods

Data presented are mean ± SD unless otherwise stated. Measurements of pulmonary blood volume, extravascular tissue density, Bmax, and MNL Bmax were compared (first versus second measurement) using a paired Student's *t* test. Baseline values for Groups 1, 2, and 3 were compared using one-way analysis of variance (ANOVA). Age, extravascular tissue density, and MNL Bmax of the groups were compared using the Kruskal-Wallis test. A nonparametric analysis was chosen because of the unequal variances. The equality of the variances was assessed using either the F test (variance ratio test for two groups) or the Schweder test (for more than two groups).

ΔSGaw was analyzed with three-way ANOVA, three factors being subject, 2-wk albuterol treatment, and acute dose used in the bronchodilator response measurements. Subject is designated as a random factor, while 2-wk albuterol treatment and albuterol dose used in the SGaw measurements were designated as fixed factors. The significance of the fixed factors was assessed against their interaction with the random factor. T tests were then performed to compare the effects of the 2-wk treatment, at each dose used in SGaw measurements, using the pooled estimate of the standard deviation from the analysis of variance. All tests were two-tailed and significance was assigned to $p < 0.05$.

RESULTS

Study Population

Eighteen male subjects were studied. The Study 1 group was 33 ± 4.1 yr old. The Study 2 group was 33 ± 2.8 yr old and one of them was a smoker. The Study 3 group was 44 ± 11.2 yr old and two of these were smokers. None of these groups differed significantly in age.

Baseline Studies

The first study in each of the 18 subjects was carried out following an identical protocol under baseline conditions. Overall (for all three groups), pulmonary blood volume was 0.15 ± 0.02 ml/ml, extravascular tissue density was 0.18 ± 0.04 g/ml, and pulmonary β-adrenoceptor density (Bmax) was 10.7 ± 1.9 pmol/g. MNL Bmax was 41.8 ± 18.2 fmol/mg protein. Individual values for the three groups are given in Table 1, both for the baseline studies and for the second measurement. There were no statistically significant differences in the baseline measurements of blood volume, extravascular tissue density, pulmonary Bmax, or MNL Bmax, between the three study groups (one-way ANOVA).

Reproducibility

In Study 1, repeat measurements of blood volume, extravascular density, pulmonary Bmax, and MNL Bmax varied by 6.9% (−4.3% to 11.8%), 7.2% (−10.7% to 15.6%), 9.9% (−15.5% to 14.1%), and 13.2% (−24.2% to 20.8%) respectively (Figure 1). The coefficient of repeatability, defined as the 95% range for the difference in two repeat measurements, was 0.02 ml/ml for blood volume, 0.03 g/ml for extravascular density, 3.07 pmol/g for pulmonary Bmax, and 13.3 fmol/mg protein for MNL Bmax.

Pulmonary Bmax Measurements for Studies 2 and 3

Study 2. Mean Bmax at the first scan was 11.0 ± 2.0 pmol/g.

TABLE 1

BLOOD VOLUME, EXTRAVASCULAR TISSUE DENSITY, AND β-ADRENOCEPTOR BINDING CAPACITY FOR LUNG TISSUE AND MONONUCLEAR LEUKOCYTES IN EACH STUDY GROUP FOR THE FIRST AND SECOND MEASUREMENT*

	Study 1 (*n* = 5)	Study 2 (*n* = 7)	Study 3 (*n* = 6)
VB1, ml/ml	0.157 ± 0.022	0.155 ± 0.014	0.143 ± 0.023
VB2, ml/ml	0.163 ± 0.018	0.154 ± 0.006	0.145 ± 0.025
DEV1, g/ml	0.153 ± 0.024	0.175 ± 0.023	0.206 ± 0.061
DEV2, g/ml	0.155 ± 0.029	0.177 ± 0.025	0.193 ± 0.053
Lung Bmax1, pmol/g	11.6 ± 2.0	11.0 ± 2.0	9.5 ± 1.5
Lung Bmax2, pmol/g	11.6 ± 1.5	8.6 ± 1.8†	9.0 ± 1.4
MNL Bmax1, fmol/mg	35.6 ± 10.3	55.1 ± 24.8‡	34.8 ± 10.5
MNL Bmax2, fmol/mg	34.7 ± 11.2	29.9 ± 13.3†‡	35.7 ± 8.1

Definition of abbreviations: 1 = first measurement; 2 = second measurement; VB = blood volume; DEV = extravascular tissue density; Bmax = maximal binding capacity; pmol/g = pmol/g tissue; fmol/mg = fmol/mg protein.

* Data are expressed as mean ± SD.

† $p < 0.05$ versus first measurements.

‡ $n = 6$.

After 2 wk of treatment with albuterol, pulmonary Bmax fell in every subject (Figure 2). The percentage reduction varied from 8% to 42%. The mean fall was 22% ± 14% (n = 7). Mean Bmax after treatment with the albuterol was 8.6 ± 1.8 pmol/g. The difference was significant ($p < 0.05$).

Study 3. Mean Bmax was 9.5 ± 1.5 pmol/g at the first scan and 9.0 ± 1.4 pmol/g at the second scan. The difference was not significant.

Bronchodilator Response

Bronchodilator response to inhaled albulterol was measured both before and after the 2-wk treatment with inhaled and oral albuterol. The response of specific airway conductance to increas-

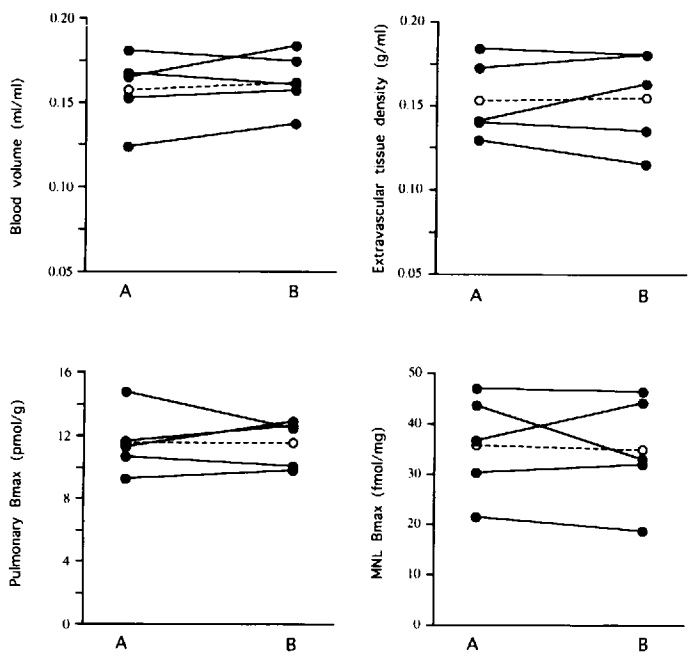

Figure 1. Reproducibility data (Study 1) for pulmonary blood volume, extravascular tissue binding capacity, pulmonary β-receptor binding capacity (Bmax), and mononuclear leukocyte Bmax, comparing PET scan 1 with, 2 wk later, PET scan 2. *Open circles*, mean values.

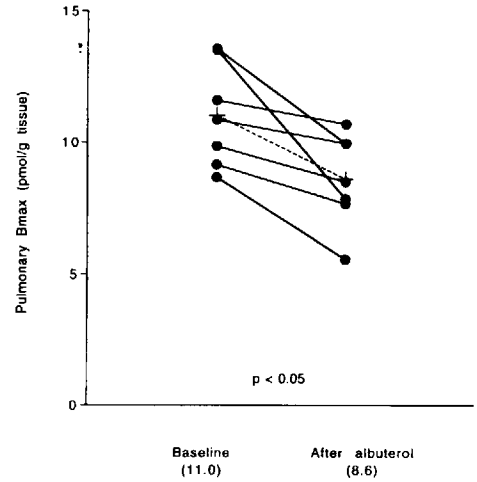

Figure 2. Changes in pulmonary adrenergic receptor binding capacity (Bmax) after 2 wk of oral and inhaled albuterol. +——+ represents the mean for the group.

ing doses of albuterol is shown in Figure 3. Baseline SGaw increased after chronic albuterol dosing, whereas ΔSGaw was reduced. A three-way ANOVA was performed, giving a p value of 0.001 for the effect of acute albuterol dosing on the ΔSGaw measurements; a p value of 0.05 for the effect of chronic albuterol therapy (before versus after); and a p value of 0.05 for the interaction between the 2-wk albuterol therapy and the four acute albuterol doses used during SGaw measurements, i.e., the effect of chronic dosing varies with acute dose. The *t* tests, using the pooled standard deviation from the ANOVA table, were therefore used to assess the effect of the chronic albuterol dosing at each individual dose point in the acute dose-response (SGaw) curve (Figure 3). The reduction in ΔSGaw was significant at the 200 μg ($p = 0.03$) and 400 μg ($p = 0.006$) dose points but did not reach significance at the 25 μg ($p = 0.12$) and 75 μg ($p = 0.06$) dose levels.

Mononuclear Leukocyte β-Adrenoceptors in Studies 2 and 3

Study 2. In the group treated for 2 wk, every subject had a fall in Bmax. The mean fall was 42% ± 19% with a range between 19% and 66%. The mean decreased from 55.1 ± 24.8 fmol/mg (excluding one subject whose second sample was lost) to 29.9 ± 13.3 fmol/mg (Figure 4). The difference was significant ($p < 0.05$).

Study 3. MNL Bmax at the first measurement was 34.8 ± 10.5 fmol/mg and 35.7 ± 8.1 fmol/mg at the time of the infusion. The difference was not significant.

Relationship between Leukocyte and Pulmonary β-Adrenoceptor Density

There was no relationship observed between MNL β-adrenoceptor Bmax and pulmonary β-adrenoceptor Bmax for the 18 subjects measured at baseline. However, in Study 2 after treatment for 2 wk with albuterol, the reduction observed in MNL Bmax was correlated ($r = 0.9$, $p < 0.05$) with the reduction in the pulmonary Bmax (Figure 5). The percentage fall in MNL Bmax was on average 2.5 ± 1.3 times the percentage fall in pulmonary Bmax.

Plasma Albuterol

In Study 3, the mean of the subjects' plasma albuterol concentrations during the albuterol infusion was 4.3 ± 1.9 μg/L. In Study 2, one of the subject's samples was lost. In the remaining six subjects, the mean plasma albuterol concentration at the time of the second scan was 6.1 ± 0.94 μg/L. There was no significant correlation between the plasma albuterol concentration during PET scan 2 and the change observed in pulmonary Bmax in either Study 2 or 3.

Plasma Catecholamines

There was no significant difference between norepinephrine or epinephrine levels measured at the first study compared with those measured at the second study. For all subjects taken together the norepinephrine and epinephrine levels at baseline measurements were 1.47 ± 0.44 and 0.28 ± 0.13 ng/ml, respectively.

DISCUSSION

In this study, we present findings which support the hypothesis that chronic β_2-agonist dosing results in downregulation of pulmonary β-adrenoceptors. This was manifest by a 22% reduction in pulmonary (Figure 2) and a 42% reduction in circulating MNL (Figure 4) β-adrenergic receptor density after chronic but not after acute albuterol dosing. The percentage change in pulmonary

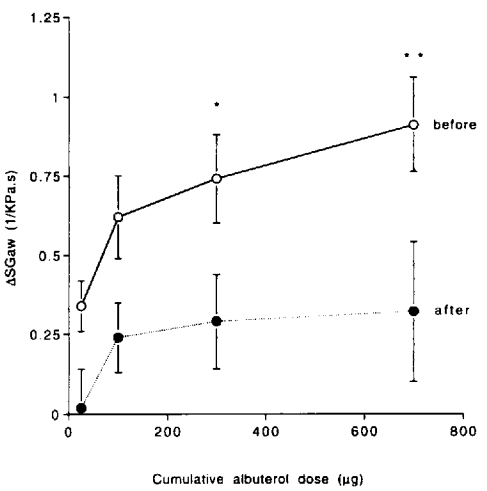

Figure 3. Changes in specific airway conductance (ΔSGaw) in response to increasing doses of inhaled albuterol before and after 2 wk of chronic dosing with oral and inhaled albuterol. *$p < 0.05$, **$p < 0.01$.

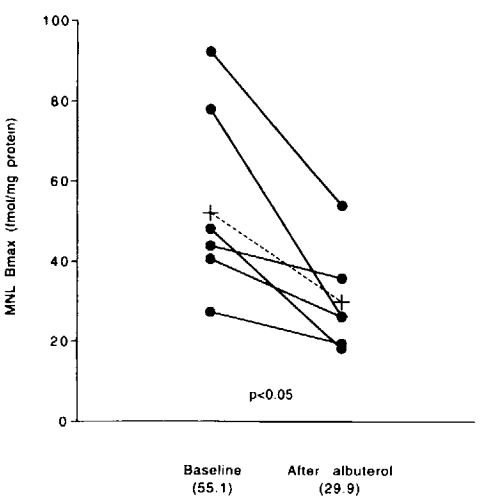

Figure 4. Changes in mononuclear leukocyte (MNL) Bmax after 2 wk of chronic dosing with oral and inhaled albuterol. +——+ represents the mean for the group.

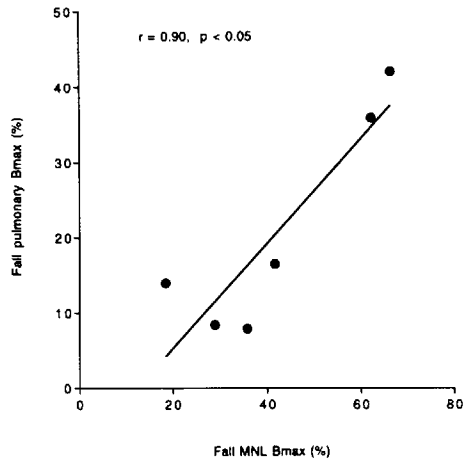

Figure 5. Relationship between percentage change in pulmonary and MNL Bmax after 2 wk of chronic dosing with oral and inhaled albuterol.

Bmax was proportional to the percentage change in MNL Bmax (Figure 5). Chronic dosing with high-dose oral and inhaled albuterol caused a significant reduction in bronchial response to acute albuterol challenge (Figure 3).

Baseline Values: Comparison with Previous Estimates

Pulmonary receptor density (Bmax) has been expressed as picomoles per gram of extravascular tissue, whereas MNL Bmax has been expressed as femtomoles per milligram of protein. An approximate conversion from one set of units to the other can be made assuming a tissue:protein ratio of 10:1 (19). In this sense, the baseline measurement of pulmonary adrenoceptor Bmax (combining Studies 1, 2, and 3), which was 10.7 ± 2.0 pmol/g (n = 18), would be equivalent to 107 fmol/mg protein. This compares well with previous measurements made *in vitro* using [^{125}I]-cyanopindolol or [^{125}I]-pindolol and human lung tissue: 126 fmol/mg protein for tissue sections (17), and 83 fmol/mg protein (26) and 95 fmol/mg protein (17) for membrane preparations. However, these values are all somewhat lower than those previously published by Hauck and coworkers (18) of 235 fmol/mg protein for lung membranes. The present study Bmax value of 10.7 pmol/g lung tissue is also in good agreement with our previous measurement using ^{3}H-CGP12177 on resected lung tissue from patients with lung cancer, which give a value of 99 fmol/mg protein (21). The baseline measurement of MNL adrenoceptor Bmax was 41.8 ± 18.2 fmol/mg protein (n = 18), which also compares well with previously reported values of 45.6 fmol/mg protein (27) and 44.2 fmol/mg protein (15).

The Bmax value of 10.7 ± 1.9 (n = 18, 36 yr [29–63]) pmol/g tissue reported for normal lung in this study is lower than our previously determined value of 14.8 ± 1.6 (n = 6, 26 yr [21–34]) pmol/g for normal lung tissue (20). This discrepancy appears to depend on the difference in extravascular density (Dev). The Dev value of 0.18 ± 0.04 obtained in the current study is higher than the value of 0.137 ± 0.015 g/ml (n = 6) reported by Ueki and coworkers (20). Table 1 (and Figure 1) shows that there is considerable intergroup and intersubject variation in Dev. Nevertheless, each group behaved consistently for Dev between the first and second scan (Table 1). Because 55 to 75% of the pixel volume is occupied by alveolar air, comparatively small variations in regional lung expansion can change Dev from 0.15 to 0.21 g/ml. In terms of Bmax per milliliter thoracic volume (or per pixel) the range of values for Groups 1, 2, and 3 in this study (1.87 to 1.92 pmol/ml) is very similar to that in our previous study (2.02 pmol/ml) (20).

It is interesting to note the difference in receptor density between lung and MNL. Converting the units of MNL receptor density to pmol/g tissue (*see above*) gives a notional Bmax value of 4.2 pmol/g tissue, which is much lower than the pulmonary Bmax of 10.7 pmol/g tissue. The reasons for this difference are not clear, but it may suggest that different tissues or cells need a different receptor density to function optimally or that different tissues have different receptor reserves (28).

Liggett and colleagues (26) studied a group of 15 patients undergoing pulmonary resection using a lipophilic ligand ([^{125}I]-pindolol. They obtained a Bmax value of 83 (32–150) fmol/mg protein for resected lung tissue and 56 (29–133) fmol/mg protein for MNLs and reported a correlation between MNL and lung β-receptor densities ($r = 0.85$, $p < 0.001$). Although their mean values were not dissimilar to ours (10.7 [7.3–14.8] pmol/g tissue for lung and 41.8 [21.3–92.3] fmol/mg protein for lymphocytes), we did not find the 5-fold intersubject variation in lung receptor density; especially, we did not observe any pulmonary Bmax values that were lower than the mean MNL Bmax for the group (i.e., our lowest lung Bmax value was 7.3 pmol/g tissue, equivalent to approximately 73 fmol/mg protein, which was higher than the mean MNL Bmax value of 41.8 fmol/gm protein). The patients they studied had an average percent predicted FEV_1 of 83% and some of them were probably long-term smokers. Strictly speaking, the resected lung tissue they obtained was not normal, although the tissue was from a region free from gross tumor. This may in part explain the difference between their findings and ours. No direct relationship between circulating MNL and lung β-receptor density was observed in the present study (n = 18). Our data do not support the use of a single measurement of circulating MNL receptor density to predict the pulmonary receptor density.

Effect of Chronic Albuterol Dosing

MNL β-adrenoceptors. MNL β-adrenoceptor density fell by 42% after 2 wk treatment with oral and inhaled β_2-agonist. Similar changes have been reported after 2 wk of oral terbutaline by Martinsson and coworkers (54%) (15) and van den Berg and coworkers (approximately 52%) (16).

Pulmonary β-adrenoceptors. Pulmonary β-adrenoceptor density fell by 22% after 2 wk of high-dose oral and inhaled albuterol. The percentage reduction varied individually from 8% to 42%. The large difference in individual response to β-agonists is noteworthy. The existence of genetic polymorphisms of the β_2-adrenergic receptors in both normal and asthmatic populations has been described (29). The Arg16 to glycine amino acid substitution confers an increased susceptibility to β-agonist-induced downregulation, while resistance to agonist-induced desensitization is associated with a Gln27 to Glu substitution. This could explain why the β-receptors in some individuals are more vulnerable to downregulation than others. The average reduction in receptor density of 22% is relatively small. However, individuals at the extreme end of the spectrum (i.e., 42% downregulation) might become relatively refractory to further β-agonist stimulation.

In a recent report, Turki and coworkers (30) harvested bronchial epithelial cells and alveolar macrophages at bronchoscopy before and after metaproterenol (10 mg) inhaled every 4 h for 24 h in eight normal subjects with the Gly16/Gly β_2-adrenergic receptor (β_2AR) genotype. Using a radioligand binding assay, a 70% decrease in β_2AR expression was found following β-agonist dosing. Our study, in the intact lung, confirms their findings in lung cells *ex vivo.* Hauck and coworkers (18) measured pulmonary β-adrenoceptor density in specimens from patients undergoing lobectomy. Patients with chronic airflow limitation were

treated preoperatively with terbutaline while patients without airflow limitation were not treated with terbutaline and acted as control subjects. They reported a 13% reduction in β-receptor density in resected lung tissue, although this did not reach statistical significance. Terbutaline was given by 0.5 mg subcutaneous injection twice daily for 2 to 3 days before the thoracotomy. They found that MNL β-adrenoceptor Bmax was 57% lower in the treated group ($p < 0.05$).

Correlation between pulmonary and MNL β-adrenoceptor changes. For all subjects, downregulation of MNL β-adrenoceptors was greater than pulmonary β-adrenoceptors. This observation may explain, at least in part, why it has been easier in the past to demonstrate downregulation on MNL cells than in lung. It also indicates that the extent of downregulation may be cell- or tissue-type dependent (28). This contention is supported by animal work (13). The correlation between the degree of downregulation seen in lung and in MNL suggests that measurement of changes in MNL Bmax may be used as a surrogate for the changes in pulmonary Bmax. However, extrapolation to other experimental conditions should be made with caution. Three important factors contribute to the relationship: direct effect of β-agonists on β-receptors, the relatively high doses used, and individual susceptibility.

Bronchodilator responses. The bronchodilator response in normal subjects becomes relatively refractory to the effects of inhaled albuterol after three (7) or four (31) weeks of treatment. Subjects in this study received higher doses of albuterol, but for a shorter period of 2 wk. Figure 3 suggests an overall trend toward tachyphylaxis with a significant difference demonstrated at the high dose levels of 200 μg and 400 μg. The difference in response at the lower dose levels of 25 μg ($p = 0.12$) and 75 μg ($p = 0.06$) did not reach significance at the 5% level. We believe this is partly due to the large variation in our SGaw measurements and the small number of subjects studied. It is also possible that larger differences might have been seen if treatment had been continued for longer, as in previous studies.

The β-receptors surveyed with this technique include both the bronchial smooth muscle and alveolar populations. Our finding that a 22% reduction in total pulmonary β-adrenoceptor density was associated with tachyphylaxis of the bronchodilator response supports the hypothesis that airway β-receptor density changes in parallel with the total pulmonary β-receptor pool. This finding also supports the use of total pulmonary β-receptors as an index for the subpopulation of β-receptors in airway smooth muscle, although the latter may be somewhat less susceptible to β-agonists, as evidenced by the presence of high levels of mRNA (32). Continuous exposure of guinea pigs to norepinephrine for a week (13) reduced β-receptor density not only in the alveoli, but also in airway smooth muscle and epithelium and in vascular smooth muscle and endothelium. The reduction in receptor number was associated with a reduction in β-receptor mRNA expression and a reduced relaxation response to β-agonist. This animal study implies that the β-agonist-induced receptor downregulation is a generalized phenomenon throughout the lung.

Reproducibility of Measurements

The results from Study 1 suggest that measurements of pulmonary and MNL β-adrenoceptor Bmax have acceptable reproducibility. The means of individual differences in the measurements of pulmonary and MNL Bmax were 9.9% and 13.2% respectively. The differences in the means for the group as a whole ($n = 5$) were much less, 0.8% for pulmonary Bmax and 2.6% for MNL Bmax. The changes seen in Study 2 of 22% for pulmonary Bmax and 42% for MNL Bmax following β-agonist therapy cannot be explained by variability in the measurement technique.

Residual Plasma Albuterol

The study design inevitably represented a compromise. A high dose of oral and inhaled albuterol was deliberately chosen to maximize the likelihood of a positive result. A placebo arm was not included in the study because of resource and radiation implications. PET scans were done 16 h after the last dose of albuterol and measures of bronchodilator response approximately 3 h after the PET scans. The samples for MNL cell Bmax measurement were taken at the beginning of the PET scan. The reason that the measurement of pulmonary β-adrenoceptor Bmax was made only 16 h after the last dose of albuterol was to minimize any "resynthesis" of receptors starting after cessation of β-agonist treatment. One study suggests that receptor density on lymphocytes may recover to normal within 3 d (14). However, a consequence of choosing a 16-h time point was that there were measurable levels of residual albuterol present in the plasma at the time of the second PET scan.

We were concerned that this residual albuterol may have interfered with the measurements of receptor density by competing with CGP-12177 for the receptors. This is very unlikely for a number of reasons. First, the *in vitro* measurement of receptor density on MNLs is highly unlikely to have been affected by residual plasma albuterol as cells were washed repeatedly to remove plasma before incubation with CGP-12177 for 1 hr. This ligand is known to have a much higher affinity for β-adrenoceptors than albuterol (33, 34). It is therefore unlikely that albuterol interfered with the binding of CGP-12177 *in vitro*. Thus, the 42% reduction in MNL Bmax in Study 2 represents receptor downregulation.

Second, in subjects treated for 2 wk, the change seen in lung β-adrenoceptors correlated well with the change seen in MNL receptors (Figure 5). Third, the change in pulmonary β-adrenoceptors did not correlate with the level of residual albuterol present at the time the measurements were made.

Finally, the acute albuterol infusion studies (to reproduce the plasma levels of residual albuterol recorded in Study 2) show that such levels of plasma albuterol do not compete significantly with CGP-12177 for occupancy of pulmonary or MNL β-adrenoceptors. The mean level of 4.3 μg/L, present during the infusions, was associated with a 4.5% reduction and a 8.6% increase in pulmonary and MNL measurements respectively. In comparison, in the group treated for 2 wk, a level of 6.1 μg/L was present at the time the measurements were made and yet a 22% reduction was seen in the lung studies and a 42% reduction in the MNL studies.

With regard to the validity of the comparison between Studies 2 and 3, short-term exposure to β-agonists for 60 min (as in Study 3) reduces agonist binding to βARs in intact cell preparations (10, 35). This is associated with a reduction in the ratio of high- to low-affinity (Kdhigh/Kdlow) βARs, probably due to receptor internalization. Because the agonist binds selectively to high-affinity βARs, receptor internalization of the high-affinity moiety might reduce albuterol binding for a given plasma concentration, allowing increased access by the antagonist, CGP12177. It is not known if selective receptor internalization of the high-affinity receptors occurs to the same extent, at the same plasma albuterol level, with the chronic β-agonist dosing of Study 2. Clearly, any substantial differences in albuterol affinity would invalidate the comparison between Study 2 and Study 3, but this is very unlikely because the plasma albuterol levels under consideration are too low (2×10^{-8} M) for significant desensitization to occur, based on the acute dosing competition curves of Toews and coworkers (35) and Su and coworkers (11).

In conclusion, with the use of positron emission tomography and an *in vitro* radioligand binding assay, we have demonstrated that 2 wk of oral and inhaled albuterol results in downregula-

tion of both pulmonary and MNL β-adrenoceptors. This was associated with a significant reduction in bronchodilator response to inhaled albuterol. The change in pulmonary β-adrenoceptors correlated well with that in MNL cells. It may be possible to predict changes in pulmonary β-adrenoceptors by measuring changes in peripheral blood MNL β-adrenoceptors. The degree of the receptor reduction varies considerably among individuals so that the problem of desensitization may be more serious for some. Extension of these studies to subjects with asthma is planned.

Acknowledgment: The writers thank A. D. Williams, A. R. K. Blyth, and the personnel of the counting laboratory, radiochemistry, and the cyclotron operations group for their assistance in performing the PET scans. The writers also thank C. Doré of the Royal Postgraduate Medical School, Medical Statistics Unit for statistical advice and help in analyzing the ΔSGaw results. This work was supported by Allen and Hanburys Ltd., the National Asthma Campaign, and the British Lung Foundation.

References

1. Jackson, R., M. R. Sears, R. Beaglehole, and H. H. Rea. 1988. International trends in asthma mortality: 1970 to 1985. *Chest* 94:914–918.
2. Grainger, J., K. Woodman, N. Pearce, J. Crane, C. Burgess, A. Keane, and R. Beasley. 1991. Prescribed fenoterol and death from asthma in New Zealand, 1981–1987: a further case-control study. *Thorax* 46:105–111.
3. Spitzer, W. O., S. Suissa, P. Ernst, R. I. Horwitz, B. Habbick, D. Cockcroft, J. F. Boivin, M. McNutt, A. S. Buist, and A. S. Rebuck. 1992. The use of beta-agonists and the risk of death and near death from asthma. *N. Engl. J. Med.* 326:501–506.
4. Sears, M. R., D. R. Taylor, C. G. Print, D. C. Lake, Q. Q. Li, E. M. Flannery, D. M. Yates, M. K. Lucas, and G. P. Herbison. 1990. Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet* 336: 1391–1396.
5. Chapman, K., S. Kesten, and J. Szalai. 1994. Regular versus as-needed albuterol in asthma control. *Lancet* 343:1379–1382.
6. Lipworth, B. J., A. D. Struthers, and D. G. McDevitt. 1989. Tachyphylaxis to systemic but not to airway responses during prolonged therapy with high dose inhaled albuterol in asthmatics. *Am. Rev. Respir. Dis.* 140:586–592.
7. Holgate, S. T., C. J. Baldwin, and A. E. Tattersfield. 1977. Beta-adrenergic agonist resistance in normal human airways. *Lancet* 2:375–377.
8. Gibson, G. J., J. K. Greenacre, P. Konig, M. E. Conolly, and N. B. Pride. 1978. Use of exercise challenge to investigate possible tolerance to beta-adrenoceptor stimulation in asthma. *Br. J. Dis. Chest* 72:199–206.
9. Cockcroft, D. W., C. P. McParland, S. A. Britto, V. A. Swystun, and B. C. Rutherford. 1993. Regular inhaled albuterol and airway responsiveness to allergen. *Lancet* 342:833–837.
10. Insel, P., L. Mahan, H. Motulsky, L. Stoolman, and A. Koachman. 1983. Time dependent decreases in binding affinity of agonists for beta adrenergic receptors of intact S49 lymphoma cells. *J. Biol. Chem.* 258:13597–13605.
11. Su, Y.-F., K. Harden, and J. Perkins. 1980. Catecholamine specific desensitization of adenylate cyclase. *J. Biol. Chem.* 255:7410–7419.
12. De Blasi, A., M. Lipartiti, H. J. Motulsky, P. A. Insel, and M. Fratelli. 1985. Agonist-induced redistribution of beta-adrenergic receptors on intact human mononuclear leukocytes: redistributed receptors are nonfunctional. *J. Clin. Endocrinol. Metab.* 61:1081–1088.
13. Nishikawa, M., J. Mak, H. Shirasaki, S. Harding, and P. Barnes. 1994. Long-term exposure to norepinephrine results in down-regulation and reduced mRNA expression of pulmonary beta-adrenergic receptors in guinea pigs. *Am. J. Respir. Cell Mol. Biol.* 10:91–99.
14. Brodde, O. E., A. Daul, M. R. Michel, F. Boomsma, A. J. Man in't Veld, P. Schlieper, and M. C. Michel. 1990. Agonist-induced desensitization of beta-adrenoceptor function in humans: subtype-selective reduction in beta 1- or beta 2-adrenoceptor-mediated physiological effects by xamoterol or procaterol. *Circulation* 81:914–921.
15. Martinsson, A., K. Larsson, and P. Hjemdahl. 1987. Studies in vivo and in vitro of terbutaline-induced beta-adrenoceptor desensitization in healthy subjects. *Clin. Sci.* 72:47–54.
16. Van de Berg, W., J. G. Leferink, J. K. Fokkens, J. Kreukniet, R. A. Maes, and P. L. Bruynzeel. 1982. Clinical implications of drug-induced desensitization of the beta receptor after continuous oral use of terbutaline. *J. Allergy Clin. Immunol.* 69:410–417.
17. Carstairs, J. R., A. J. Nimmo, P. J. Barnes. 1985. Autoradiographic visualization of beta-adrenoceptor subtypes in human lung. *Am. Rev. Respir. Dis.* 132:541–547.
18. Hauck, R. W., M. Bohm, S. Gengenbach, P. L. Sunder, G. Fruhmann, and E. Erdmann. 1990. Beta 2-adrenoceptors in human lung and peripheral mononuclear leukocytes of untreated and terbutaline-treated patients. *Chest* 98:376–381.
19. Delforge, J., A. Syrota, J.-P. Lancon, K. Nakajima, C. Loc'h, M. Janier, J. Vallois, J. Cayla, and C. Crouzel. 1991. Cardiac beta-adrenergic receptor density measured in vivo using PET, CGP12177, and a new graphical method. *J. Nucl. Med.* 32:739–748.
20. Ueki, J., C. G. Rhodes, J. M. B. Hughes, R. De Silva, D. Lefroy, P. W. Ind, F. Qing, F. Brady, S. K. Luthra, C. Steel, S. L. Waters, A. A. Lammertsma, P. G. Camici, and T. Jones. 1993. In vivo quantification of pulmonary β-adrenoceptor density in humans with (*S*)-[^{11}C]-CGP-12177 and PET. *J. Appl. Physiol.* 75:559–565.
21. Qing, F., C. G. Rhodes, M. J. Hayes, T. Krausz, S. W. Fountain, T. Jones, and J. M. B. Hughes. *In vivo* quantification of human pulmonary β-adrenoceptor density using PET: comparison with *in vitro* radioligand binding. *J. Nucl. Med.* (In press)
22. Rhodes, C. G., P. Wollmer, F. Fazio, and T. Jones. 1981. Quantitative measurement of regional extravascular lung density using positron emission and transmission tomography. *J. Comput. Assist. Tomogr.* 5:783–791.
23. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Lab. Clin. Invest.* 21(Suppl. 97):77–89.
24. McCarthy, P. T., S. Atwal, A. P. Sykes, and J. G. Ayres. 1993. Measurement of terbutaline and albuterol in plasma by high performance liquid chromatography with fluorescence detection. *Biomed. Chromatogr.* 7:25–28.
25. Lefroy, D. C., R. de Silva, L. Choudhury, N. G. Uren, T. Crake, C. G. Rhodes, A. A. Lammertsma, H. Boyd, P. N. Patsalos, P. Nihoyannopoulos, C. M. Oakley, T. Jones, and P. G. Camici. 1993. Diffuse reduction of myocardial beta-adrenoceptors in hypertrophic cardiomyopathy: a study with positron emission tomography. *J. Am. Coll. Cardiol.* 22:1653–1660.
26. Liggett, S. B., J. C. Marker, S. D. Shah, C. L. Roper, and P. E. Cryer. 1988. Direct relationship between mononuclear leukocyte and lung β-adrenergic receptors and apparent reciprocal regulation of extravascular, but not intravascular, α- and β-adrenergic receptors by the sympathochromaffin system in humans. *J. Clin. Invest.* 82:48–56.
27. Connolly, M., J. Crowley, C. Nielson, N. Charan, and R. Vestal. 1994. Peripheral mononuclear leucocyte beta adrenoceptors and non-specific bronchial response to methacholine in young and elderly normal subjects and asthmatic patients. *Thorax* 49:26–32.
28. Barnes, P. J. 1995. Beta-adrenergic receptors and their regulation. *Am. J. Respir. Crit. Care Med.* 152:838–860.
29. Reihaus, E., M. Innis, N. MacIntyre, and S. B. Ligget. 1993. Mutations in the gene encoding for the β_2-adrenergic receptor in normal and asthmatic subjects. *Am. J. Respir. Cell Mol. Biol.* 8:334–339.
30. Turki, J., S. A. Green, K. B. Newman, M. A. Meyers, and S. B. Liggett. 1995. Human lung cell β2-adrenergic receptors desensitize in response to in vivo administered β-agonist. *Am. J. Physiol.* 269 (*Lung Cell Mol. Physiol.* 13):L709–L714.
31. Harvey, J. E., and A. E. Tattersfield. 1982. Airway response to salbutamol: effect of regular salbutamol inhalations in normal, atopic, and asthmatic subjects. *Thorax* 37:280–287.
32. Hamid, Q. A., J. C. W. Mak, M. N. Sheppard, B. Corrin, J. C. Venter, and P. J. Barnes. 1991. Localization of β_2-adrenoceptor messenger RNA in human and rat lung using in situ hybridization: correlation with receptor autoradiography. *Eur. J. Pharmacol. (Molec. Pharmacol.)* 206:133–138.
33. Law, M. 1993. Demonstration of the suitability of CGP 12177 for in vivo studies of β-adrenoceptors. *Br. J. Pharmacol.* 109:1101–1109.
34. van Koppen, C., M. Hermanussen, K. Verrijp, J. F. Rodrigues de Miranda, A. Beld, J. Lammers, and C. van Ginneken. 1987. β-adrenoceptors in human tracheal smooth muscle: characteristics of binding and relaxation. *Life Sci.* 40:2561–2570.
35. Toews, M. L., T. K. Harden, and J. P. Perkins. 1983. High-affinity binding of agonists to β-adrenergic receptors on intact cells. *Proc. Natl. Acad. Sci. U.S.A.* 80:3553–3557.