# Effect of long-term $\beta_2$-agonist dosing on human cardiac β-adrenoceptor expression in vivo: Comparison with changes in lung and mononuclear leukocyte β-receptors

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*Background.* **Tachyphylaxis to the cardiac effects of β-adrenoceptor stimulation after long-term $\beta_2$-agonist administration is well recognized, but the influence on global cardiac β-adrenoceptor density has not been previously investigated in vivo. Positron emission tomography (PET) has made possible the noninvasive quantification of regional receptor density. This study assesses the effect of long-term $\beta_2$-agonist dosing on cardiac β-adrenoceptors.**

*Methods and Results.* **β-Adrenoceptors in the hearts of 29 healthy male subjects aged 35 ± 8 years were imaged and quantified in vivo by means of PET and compared with the receptor density in the same subjects' lung tissue. Mononuclear leukocyte (MNL) β-receptor density was determined in vitro by means of a radioligand binding assay. β-Receptor density was 8.41 ± 2.03 pmol/gm tissue in heart, 10.81 ± 1.91 pmol/gm tissue in lung, and 38.0 ± 17.5 fmol/mg protein on MNLs. There was a weak relationship between cardiac and pulmonary β-receptor densities ($r = 0.45$, $p < 0.02$) but not between cardiac and MNL receptor density. In seven subjects, the measurements were repeated after 2 weeks of albuterol treatment (4 mg orally twice daily and 200 μg inhaled four times daily in the first week, with doubling of the dose during the second week). After the albuterol treatment, β-receptor density fell on average by 19% ($p < 0.05$) in the heart compared with 22% ($p < 0.05$) in the lung and 42% ($p < 0.05$) in MNLs. Correlations were found between the percentage changes in receptor density in heart and lung ($r = 0.98$, $p < 0.001$) and in heart and MNLs ($r = 0.99$, $p < 0.002$).**

*Conclusions.* **Two weeks of high-dose albuterol results in equivalent downregulation of β-receptors in vivo, both in the lung and in the heart. (J Nucl Cardiol 1997;4:532-8.)**

**Key Words: β-adrenergic receptors • β-adrenergic agonists • positron emission tomography • human heart**

Inhaled selective $\beta_2$-adrenoceptor agonists are the most potent and most rapidly acting bronchodilators in current use, and they are also the most widely prescribed antiasthma treatment. Almost since their introduction, however, there has been concern, highlighted in recent studies, that regular use of inhaled $\beta_2$-agonist drugs may be associated with poor asthma control,[1] increased morbidity,[1] and increased risk of death from asthma.[2,3] Several mechanisms have been suggested as possible explanations; a prime candidate is the downregulation of $\beta_2$-adrenergic receptors by $\beta_2$-agonists.

β-Adrenoceptors are widely distributed in the human body, with $\beta_1$ subtype dominance in the heart[4] and $\beta_2$ dominance in the lung.[5,6] Mononuclear leukocyte (MNL) preparations from blood (mainly lymphocytes) have been much studied as a readily available source of human β-adrenergic receptors, all of which are of the $\beta_2$ subtype. A reduction in $\beta_2$-adrenoceptor number after long-term administration of $\beta_2$-agonists has been repeatedly demonstrated for human beings by means of circulating lymphocytes[7,8] and in vitro radioligand binding assays. It has also been shown to occur in human

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myometrium. However, there have been no in vivo studies examining change in cardiac β-receptor density (Bmax) after long-term $\beta_2$-agonist therapy. Nevertheless, cardiac β-receptor downregulation in congestive heart failure, thought to be related to the increased level of endogenous β-agonists—high catecholamine drive—has frequently been reported in studies of tissue in vitro.[9] Furthermore, tachyphylaxis to the cardiovascular effects of $\beta_2$-agonists after long-term β-agonist dosing has been demonstrated,[10] although there has been no direct measurement of cardiac Bmax.

In a previous study[11] with positron emission tomography (PET), we reported a moderate reduction (22%) in Bmax in lung after 2 weeks of albuterol dosing. This was associated with a small but significant reduction in bronchodilator response and accompanied by a larger Bmax reduction (42%) in peripheral MNLs.

In this study, we examined the hypothesis that decreased cardiac β-receptor responsiveness after long-term $\beta_2$-agonist therapy could be explained by a reduced number of cell-surface receptors. We measured cardiac Bmax in vivo before and after 2 weeks of albuterol therapy by means of the hydrophilic β-receptor ligand CGP-12177 (a nonselective β-antagonist labeled with $^{11}C$) and PET. In addition, the changes in Bmax in heart tissue were compared with the changes in lung tissue and MNLs to study individual tissue susceptibilities to β-agonists and possible relationships among the three tissues surveyed.

## METHODS

**Subjects and Treatment.** All subjects were healthy volunteers recruited locally. Subjects with any history of significant respiratory or cardiovascular illness were excluded. All subjects gave written informed consent to the protocol, which was approved by the Hammersmith Hospital Research Ethics Committee and the United Kingdom Administration of Radioactive Substances Advisory Committee.

Twenty-nine healthy male volunteers, aged 35 ± 8 years, were investigated at baseline. Six subjects, aged 30 ± 2 years, had measurements on two occasions 2 weeks apart without any intervention to assess the reproducibility of the techniques. In seven of the subjects, aged 33 ± 3 years, measurements were repeated after 2 weeks of regular treatment with high-dose oral and inhaled albuterol (known as *salbutamol* in Europe). For the first week, subjects received 200 μg inhaled albuterol four times daily and 4 mg slow-release albuterol (Volmax) orally twice daily. During the second week, subjects received 400 μg inhaled albuterol four times daily and 8 mg slow-release albuterol orally twice daily. Treatment was stopped 16 hours before measurement of cardiac, pulmonary, and MNL β-adrenoceptors. In a previous study,[11] we demonstrated that the residual level of albuterol present 16 hours after the last medication, at the time when PET scanning was started, was insufficient to interfere with the measurement of Bmax by competition with the ligand CGP-12177.

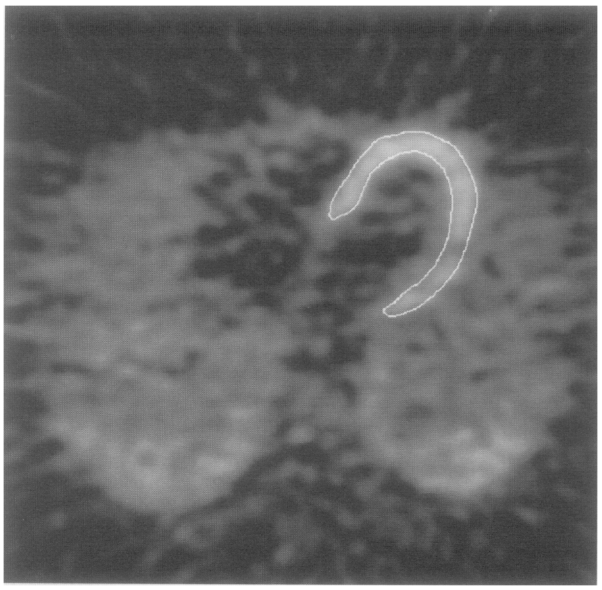

**Figure 1.** A representative PET image of β-adrenoceptor binding obtained from a healthy subject by adding the dynamic time frame images recorded between 10 and 30 minutes after the first *(S)*-[$^{11}$C]CGP-12177 injection. ROIs for heart (left ventricular wall and septum) were drawn on this image.

**Measurement of Cardiac and Pulmonary Bmax.** The preparation of the *(S)*-[$^{11}$C]CGP-12177, the PET scanning, and the calculation of Bmax, were performed as previously reported.[12,13] A nonselective hydrophilic β-antagonist, *(S)*-CGP-12177, was used as the β-receptor ligand in all studies. This was labeled with the positron-emitting radionuclide $^{11}C$, which has a half-life of 20.4 minutes. PET scans were performed with an ECAT 931-08/12 15 plane PET scanner (Siemens/CTI, Knoxville, Tenn.). The protocol comprised (1) transmission, (2) [$^{15}$O]carbon monoxide emission, and (3) *(S)*-[$^{11}$C]CGP-12177 dynamic emission scanning, to provide image attenuation factors, region of interest (ROI) definition, and the calculation of blood volume and Bmax, respectively. Images were analyzed on Sun workstations (Sun Microsystems, Inc., Palo Alto, Calif.) by use of Analyze Image Analysis[14] and the Matlab mathematic software package (The MathWorks, Inc., Natick, Mass.). A single ROI for the heart (left ventricular wall and septum) was drawn on summed dynamic images (Figure 1), which were obtained by adding the dynamic time frame images recorded between 10 and 30 minutes after the first *(S)*-[$^{11}$C]CGP-12177 injection. The purpose of summing the dynamic time frames was to improve the signal-to-noise ratio and the visual appearance, making it easier to draw the ROIs. The Bmax surveyed in the ROIs was calculated with a graphic approach derived from the work of Delforge et al.[15] This technique relies on the relationship between Bmax and the rate of uptake of ligand into the ROI. Two injections of *(S)*-[$^{11}$C]CGP-12177 were given during the dynamic scan, and the rates of uptake were used to solve this relationship for Bmax. This technique does not provide a value for the

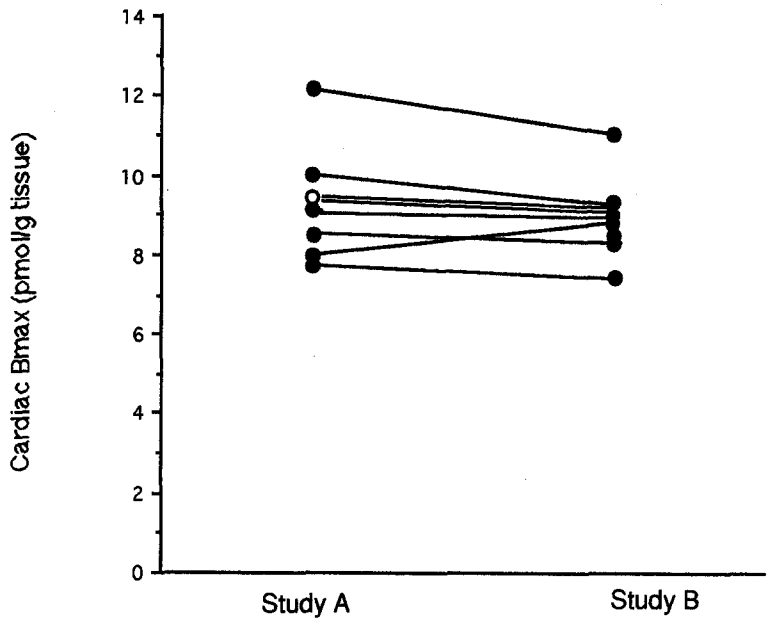


**Figure 2.** Reproducibility data for cardiac Bmax comparing PET scan 1 with PET scan 2 performed 2 weeks later. *Filled circles with solid lines* represent individual data points; *open circles with dashed lines* represent the mean for the group.

dissociation constant, and the measurement of Bmax with this method is independent of binding affinity. In a previous study, we showed that this technique has acceptable reproducibility.[11]

Vascular density (grams of blood per milliliter of thorax) was obtained by multiplying blood volume[16] (milliliters of blood per milliliter of thoracic volume) by 1.06 (whole-blood density in grams per milliliter). Heart density (blood and extravascular tissue) obtained from the normalized transmission scan[16] was expressed in grams per milliliter of thoracic volume. Extravascular tissue density (grams per milliliter) was calculated by subtracting vascular density from the heart density scans. Bmax was expressed in picomoles per gram of extravascular tissue.

**Measurement of MNL Bmax.** Preparation of MNLs and radioligand binding assay were performed as previously described.[11,13] Within each experiment, measurements were carried out in duplicate at each concentration of *(S)*-[$^3$H]CGP-12177. MNL membranes (50 to 100 μg protein) were incubated with seven concentrations (0.06 to 3.2 nmol/L) of *(S)*-[$^3$H]CGP-12177 (53 Ci/mmol; Amersham, England) at 37° C for 60 minutes in a total volume of 500 μl. The reaction was stopped by adding 2 ml ice-cold washing buffer containing 10 mmol/L tris(hydroxymethyl)aminomethane (Tris), 2 mmol/L magnesium chloride, and 0.9% sodium chloride at pH 7.4, with immediate filtration through Whatman GF/C filters (Whatman Inc., Clifton, N.J.) by means of a Brandell cell harvester (Brandell Biomedical Research and Development Laboratories, Gaithersburg, Md.). Each filter was washed with 5 ml ice-cold washing buffer three times to separate bound ligand from free. Filters with retained radioactivity were left overnight in 10 ml scintillant (Hionic-Flour; Packard Instrument Co., Meriden, Conn.) and then counted with a liquid scintillation counter (Beckman LS 6800; Beckman Instruments, Inc., Fullerton, Calif.). Protein was determined according to the procedure of Lowry et al.[17] 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. β-Adrenoceptor binding capacity (Bmax) is expressed in femtomoles per milligram protein.

**Missing Data.** Five MNL samples, including one sample from one of the seven subjects receiving albuterol, were lost when a freezer failed.

**Statistical Analysis.** Data are presented as mean ± standard deviation unless otherwise stated. Measurements were compared with the paired Student's *t* test. All tests were two-tailed, and significance was assigned to a *p* value less than 0.05.

## RESULTS

**Baseline Values.** The first study in each of the 29 subjects was carried out according to an identical protocol under baseline conditions. Extravascular tissue density was 0.71 ± 0.05 g/ml for the heart and 0.17 ± 0.04 g/ml for the lung. Cardiac Bmax was 8.41 ± 2.03 pmol/gm. Pulmonary Bmax was 10.81 ± 1.91 pmol/gm. MNL Bmax was 38.0 ± 17.5 fmol/mg protein. For the group of seven subjects who received albuterol for 2 weeks, mean baseline cardiac Bmax was 9.54 ± 2.30 pmol/gm.

**Reproducibility.** In the six subjects investigated for reproducibility, the second measurements of cardiac Bmax were 2.5%, 3%, 5%, 6.5%, and 8% higher and 9% lower than the first measurements. The mean of individual absolute (unsigned) differences was 5.7%. (Figure 2).

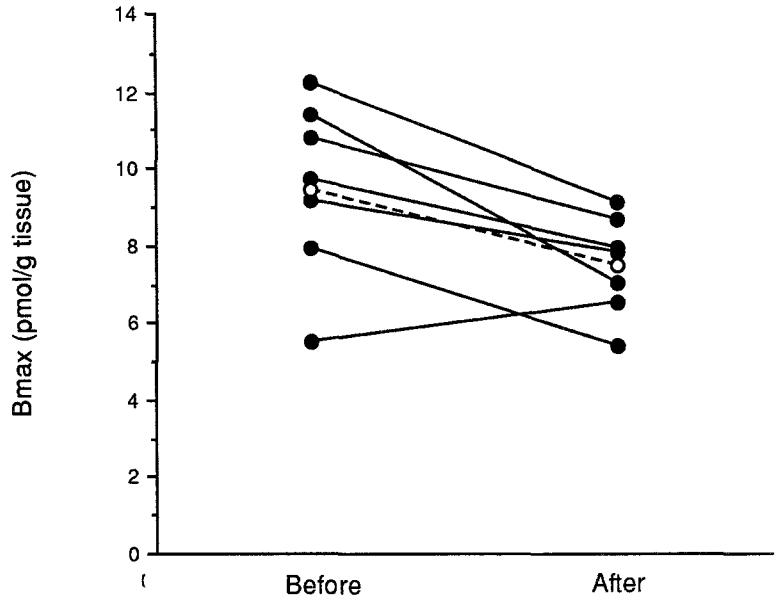


**Figure 3.** Changes in cardiac Bmax after 2 weeks of oral and inhaled albuterol. *Filled circles with solid lines* represent individual data points; *open circles with dashed lines* represent the mean for the group.

The mean value for the group as a whole ($n = 6$) changed slightly, from 9.09 to 9.00 pmol/gm, a difference of only 1% ($p$ not significant on a paired $t$ test).

**Changes in Bmax After Long-Term Albuterol Dosing.** After the 2 weeks of therapy, cardiac Bmax fell in all but one subject (Figure 3). No explanation could be found for the anomalous result in this one subject; pulmonary Bmax changes were as expected and plasma catecholamine levels were normal. The percentage reductions in the other six subjects varied from 14% to 38%, with a mean of 19%. For all seven subjects, mean Bmax after treatment was 7.50 ± 1.28 pmol/gm, which was significantly lower than the baseline value ($p < 0.05$). This 19% change in the Bmax of the heart is not dissimilar to our previous findings in the lung.[11] In that earlier report, pulmonary Bmax fell in every subject after 2 weeks of albuterol therapy. Percentage reduction varied from 8% to 42%, with an average fall of 22% ($p < 0.05$). This contrasted with a mean reduction of 42% (varying individually from 19% to 62%) in MNL Bmax ($p < 0.05$).[11]

**Relationships Between Cardiac, Pulmonary, and MNL Bmax.** At baseline, as previously reported for a smaller group,[11] there was no relationship between pulmonary and MNL Bmax ($n = 24$). Similarly, there was no relationship between cardiac Bmax and MNL Bmax at baseline ($n = 24$). However, there was a weak correlation between the cardiac Bmax and pulmonary Bmax ($r = 0.45$, $p < 0.002$, $n = 29$).

In subjects who received albuterol for 2 weeks, the percentage reduction observed in cardiac Bmax correlated with the percentage reduction in pulmonary Bmax ($r = 0.98$, $p < 0.001$, $n = 6$), excluding the lone subject with an increase in cardiac Bmax (Figure 4, *A*). Moreover, there was a correlation between the percentage reduction in MNL Bmax and the percentage reduction in cardiac Bmax ($r = 0.99$, $p < 0.02$, $n = 5$), again excluding the point with an increase in cardiac Bmax (Figure 4, *B*). This was similar to our previously reported finding of a correlation between percentage reduction in MNL Bmax and pulmonary Bmax.[11] The ratios for percentage decrease in Bmax relative to the heart were 1.2 in lung and 2.2 in MNLs.

## DISCUSSION

**Comparison With Previous Estimates.** Cardiac and pulmonary Bmax values were expressed in picomoles per gram of extravascular tissue, whereas MNL Bmax was expressed in femtomoles per milligram of protein. An approximate conversion from one set of units to the other can be made,[15] assuming a tissue to protein ratio of 10:1. Therefore, the baseline measurement of cardiac β-adrenoceptor Bmax of 8.41 ± 2.03 pmol/gm would be equivalent to about 84 ± 20 fmol/mg protein. This compares well with previous measurements made in vitro with [$^{125}$I]cyanopindolol or [$^{3}$H]CGP-12177 in nonfailing human left ventricular tissue obtained from prospective cardiac transplant donors (all figures mean ± standard error of the mean): 79 ± 3 fmol/mg protein ($n = 3$) by Stiles et al.,[18] 88 ± 7 fmol/mg protein ($n = 12$) by Bristow et al.,[19] and 93 ± 4 fmol/mg protein ($n =$

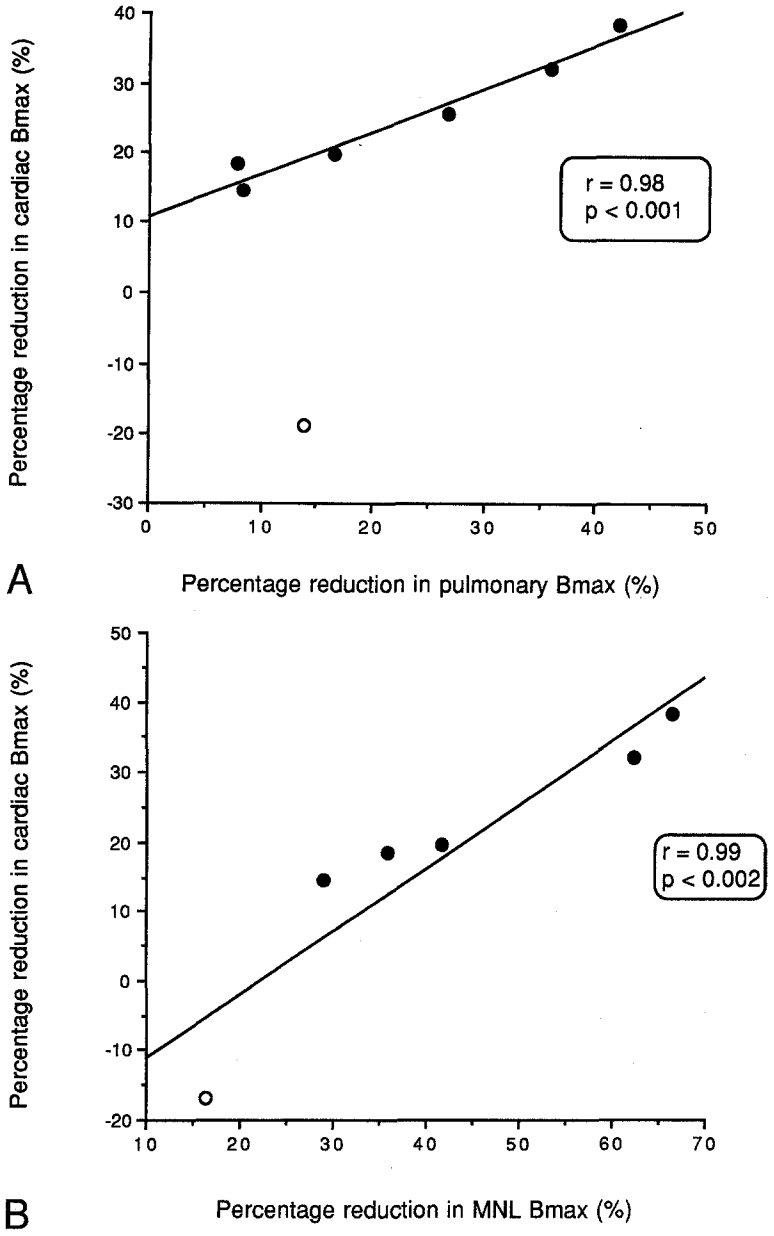


**Figure 4. A,** Relationship between percentage change in cardiac and pulmonary Bmax after 2 weeks of long-term dosing with oral and inhaled albuterol. The *open circle* denotes the subject with an increase in cardiac Bmax, excluded from the correlation statistics. B, Relationship between percentage change in cardiac and MNL Bmax after 2 weeks of long-term dosing with oral and inhaled albuterol. The *open circle* denotes the subject with an increase in cardiac Bmax, excluded from the correlation statistics.

3) by Böhm et al.[20] The Bmax value in this study (8.41 ± 2.03 pmol/gm tissue) is somewhat lower than the value of 11.50 ± 2.18 pmol/gm tissue obtained with PET in a smaller group of eight young (28 ± 7 years), healthy men reported on by Lefroy et al.[21] Baseline pulmonary adrenoceptor Bmax was 10.81 ± 1.91 pmol/gm tissue in our study ($n$ = 29), in good agreement with our previously reported[11] value of 10.7 ± 1.9 pmol/gm tissue ($n$ = 18). Baseline MNL adrenoceptor Bmax was 38.0 ± 17.5 fmol/mg protein ($n$ = 24), which is not dissimilar to previously reported values of 45.6 and 44.2 fmol/mg protein.[10,22]

**Relationships Between Cardiac, Pulmonary, and MNL β-Receptors.** Bmax varies in different tissues in vivo in human beings. Among the tissues surveyed, the Bmax ratios for heart and lung relative to MNLs are 2.3 and 2.9. These ratios support the hypothesis that different tissues and cells need different receptor

densities to function optimally; that is, that different tissues require different receptor reserves.

The MNL contains exclusively $\beta_2$-adrenoceptors, coupled to adenylate cyclase. Because they are readily available, MNLs have been used extensively as a model to study the status of β-receptors in less accessible organs and tissues such as lung (predominantly $\beta_2$) and heart (predominantly $\beta_1$). Nevertheless, we did not observe a close correlation. Peripheral MNLs contain lymphocytes and monocytes, with lymphocytes themselves being composed of different subsets that are reported to have different β-receptor densities.[23] This subset composition can be altered by a number of circumstances, including transient increases in plasma catecholamine levels.[24] These factors prevent MNLs from acting as a surrogate for other tissues. The absence of a direct relationship between peripheral MNL (exclusively $\beta_2$) and cardiac (predominantly $\beta_1$) Bmax values does not support the use of a single determination of circulating MNL Bmax to predict cardiac Bmax.

**Reproducibility of Measurements.** In an earlier study of five healthy subjects, we demonstrated that measurements of pulmonary and MNL β-receptor Bmax have acceptable reproducibility.[11] The six cardiac studies analyzed in this article showed an average of 5.7% difference individually for the two separate measurements of cardiac Bmax (Figure 2). The group mean difference was only 1%. The individual reduction of 14% to 38% in the six subjects after 2 weeks of albuterol therapy cannot therefore be explained simply by variability in the measurement technique.

**Effect of Long-Term Albuterol Dosing.** Cardiac Bmax fell on average by 19% after 2 weeks of high-dose oral and inhaled albuterol. Among the seven subjects studied, Bmax fell in six but increased in one subject, who also had the lowest baseline value of 5.50 pmol/gm tissue (Figure 2). The reason for the different behavior in this subject is unclear, but it is possible that some protective mechanisms may exist to prevent further downregulation of β-receptors by exogenous β-agonists if baseline Bmax is already low. Regional differences in cardiac Bmax were not sought. In an earlier study with similar techniques,[21] no Bmax differences were found in four myocardial ROIs (anterior, lateral, inferoposterior, and septal).

There was a significant correlation between the percentage reduction in Bmax in heart and MNLs, as well as between heart and lung, similar to that reported earlier between lung and MNLs.[11] These findings suggest that measurement of *changes* in MNL Bmax could be used as a surrogate for *changes* in cardiac and pulmonary Bmax under some circumstances. Nevertheless, extrapolation from such a correlation should be made with caution. The magnitude of the reduction in Bmax varies with tissue and is less in heart (19%) and lung (22%) than in MNLs (42%). Downregulation is a general phenomenon, and it is not clear why MNLs are more susceptible. This is unlikely to relate to the $\beta_2$-agonist concentration "seen" by the receptors, as both pulmonary and cardiac receptors would have been exposed to albuterol concentrations at least as high as those for the MNL in view of the way that the albuterol was administered in this study. We deliberately elected to administer extremely high doses of oral and inhaled albuterol to these healthy subjects. The implication is that a weaker or shorter-lasting β-adrenergic stimulus might produce downregulation of MNL $\beta_2$-receptors alone, leaving the more resistant tissues unaffected. The correlation between downregulation in different tissues and the variation in degree from person to person may reflect individual tissue susceptibility.

This study provides the first direct evidence of cardiac β-receptor downregulation in vivo after long-term β-agonist dosing. Tachyphylaxis to the cardiac effects of β-receptor stimulation after $\beta_2$-agonist administration is well recognized,[10] despite the fact that $\beta_1$-adrenoceptors predominate in the heart. Functional responses of blood pressure and heart rate to acute albuterol challenge before and after long-term dosing were not measured in this study; in a subsequent study, with a similar protocol, significant tachyphylaxis was observed for both blood pressure and heart rate.[25] It is believed that the β-receptor reserve in heart is small.[4] Nearly the whole of the receptor pool has to be occupied to achieve a maximal response. Thus, a small decrease in cardiac receptors could reduce functional responsiveness. The decreases in receptor number and in functional responsiveness in human heart failure,[4] related to high circulating catecholamine concentrations, also support this contention. Downregulation of cardiac β-receptors can be regarded as a protective response to attenuate the adverse effects of excess β-receptor stimulation, whether by endogenous agents or β-agonist drugs. In this study, tolerance to tachycardia occurred in all subjects in 3 to 4 days.

Because CGP-12177 is a nonselective antagonist, the $\beta_2$-selective agonist albuterol may have downregulated the $\beta_2$-receptors only or both the $\beta_2$ and $\beta_1$ subtypes. In vitro dissociation constant measurements for albuterol would suggest that it is relatively selective for $\beta_2$-receptors. On the other hand, the 19% downregulation of Bmax is unlikely to stem from a fall of 63% in the $\beta_2$ subpopulation alone (approximately 30% of the total cardiac β-receptors are $\beta_2$). Some "cross talk" between receptor subtypes cannot be excluded.

**Conclusions.** Through the in vivo use of hydrophilic β-receptor ligand *(S)*-[$^{11}$C]CGP-12177 and PET, 2 weeks of oral and inhaled albuterol was found to result in

downregulation of cardiac β-adrenergic receptors in human beings. The changes in cardiac Bmax correlated well with the changes in Bmax in lung and MNLs, as did changes in lung and MNLs reported previously.[11] It may be possible to predict changes in cardiac and pulmonary β-adrenoceptors by measuring changes in circulating MNL β-adrenergic receptors. At baseline, neither cardiac nor pulmonary Bmax correlated with that of MNLs, suggesting that a single determination of peripheral MNL Bmax cannot predict adrenergic Bmax in the heart or lung. Further work is required to elucidate in vivo susceptibility of the β-receptors of different tissues to downregulation.

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