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

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Background. Tachyphylaxis to the cardiac effects of $\beta$-adrenoceptor stimulation after long-term $\beta_2$-agonist administration is well recognized, but the influence on global cardiac $\beta$-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 $\beta$-adrenoceptors.

Methods and Results. $\beta$-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) $\beta$-receptor density was determined in vitro by means of a radioligand binding assay. $\beta$-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 $\beta$-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 $\mu$g inhaled four times daily in the first week, with doubling of the dose during the second week). After the albuterol treatment, $\beta$-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 $\beta$-receptors in vivo, both in the lung and in the heart. (J Nucl Cardiol 1997;4:532-8.)

Key Words: $\beta$-adrenergic receptors • $\beta$-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, increased morbidity, and increased risk of death from asthma. Several mechanisms have been suggested as possible explanations; a prime candidate is the downregulation of $\beta_2$-adrenergic receptors by $\beta_2$-agonists.

$\beta$-Adrenoceptors are widely distributed in the human body, with $\beta_1$ subtype dominance in the heart and $\beta_2$ dominance in the lung. Mononuclear leukocyte (MNL) preparations from blood (mainly lymphocytes) have been much studied as a readily available source of human $\beta$-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 and in vitro radioligand binding assays. It has also been shown to occur in human
myometrium. However, there have been no in vivo studies examining change in cardiac β-receptor density (Bmax) after long-term β₂-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. Furthermore, tachyphylaxis to the cardiovascular effects of β₂-agonists after long-term β-agonist dosing has been demonstrated, although there has been no direct measurement of cardiac Bmax.

In a previous study 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 β₂-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 ¹²⁴C) 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 β₂-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 β₂-adrenoceptors. In a previous study, 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.

**Measurement of Cardiac and Pulmonary Bmax.** The preparation of the (S)-¹²⁴C]CGP-12177, the PET scanning, and the calculation of Bmax, were performed as previously reported. 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 ¹²⁴C, 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) [¹⁵O]carbon monoxide emission, and (3) (S)-¹²⁴C]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 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)-¹²⁴C]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. This technique relies on the relationship between Bmax and the rate of uptake of ligand into the ROI. Two injections of (S)-¹²⁴C]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 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)-¹²⁴C]CGP-12177 injection. ROIs for heart (left ventricular wall and septum) were drawn on this image.](image-url)