β₂-Adrenoceptors in Human Lung and Peripheral Mononuclear Leukocytes of Untreated and Terbutaline-treated Patients*

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β₂-adrenoceptor agonists act against bronchoconstriction by stimulating β₂-adrenoceptors in bronchial smooth muscle. However, tachyphylaxis has been argued to occur because of β₂-adrenoceptor down-regulation following therapy with β₂-adrenergic agents. To investigate receptor alterations, human peripheral mononuclear leukocytes are frequently used, since human lung tissue is not easily available. In order to study whether β₂-adrenoceptors in MNL reliably reflect the conditions in the human lung tissue, we compared MNL and human lung tissue of 18 patients who had to undergo lung resection. Ten patients were untreated, and eight had bronchodilator therapy prior to therapy with terbutaline because of bronchoconstriction. Both in human lung and MNL, the β₂-adrenoceptor subpopulation was characterized by competition experiments with the β₂-selective antagonist CGP 207.12 A and the β₂-selective antagonist ICI 118.551. In MNL, a significant decrease in the density of β₂-adrenoceptors was found in treated but not in untreated patients, while the antagonist affinity of the β₂-adrenoceptors remained unchanged. However, in lung parenchyma, which was obtained at the very same time from the same patients, no down-regulation of the total amount of β₂-adrenoceptors could be measured. It is concluded that MNLs are a reliable model for studying properties of β₂-adrenoceptor regulation. However, the hereby obtained results show that MNLs do not reflect the conditions of β₂-adrenoceptors in human lung tissue. Human lung tissue is found to be less susceptible than human MNL for β₂-adrenoceptor down-regulation by terbutaline treatment at therapeutic doses. The lack of β₂-adrenoceptor down-regulation provides a biochemical explanation for the preserved efficacy of β₂-sympathomimetics after long-term antiasthmatic treatment. (Chest 1990; 98:376-81)

MNL = mononuclear leukocytes; I-CYP = iodocyanopindolol

The role of β₂-adrenoceptor agonists in the treatment of bronchial asthma is evidenced by the good reversibility of an increased airway resistance after application of β₂-sympathomimetic drugs. However, it has been argued that the efficacy of β₂-adrenoceptor agonist treatment would be limited by down regulation of lung β₂-adrenoceptors.

Peripheral mononuclear leukocytes have been suggested to provide a model to study properties of β₂-adrenoceptors because they are easily available and possess β₂-adrenoceptors coupled to adenylate cyclase. The alterations of β₂-adrenoceptors in MNL often have been used as a model to draw conclusions for alterations of the receptor density (Bmax) and coupling in solitary organs like lung or uterus.

Indeed, after treatment with the bronchodilator terbutaline, down-regulation of lymphocyte β₂-adrenoceptors has been observed. However, it has not been conclusively shown that alterations in MNL reflect the situation in the human lung parenchyma during β₂-adrenoceptor agonist treatment at therapeutic doses.

The question in the present study was whether human lung β₂-adrenoceptors are altered during anti-obstructive treatment with terbutaline. Moreover, it was studied whether or not β₂-adrenoceptors in human lung tissue reveal comparable alterations during bronchodilator treatment like in MNL. Therefore, β₂-adrenoceptor density and affinity were measured with radioligand-binding techniques in human peripheral lung from patients undergoing lung resection because of bronchial carcinoma. Patients with bronchial obstruction, as evidenced in the pulmonary function testing, were treated with the β₂-adrenoceptor agonist, terbutaline, preoperatively. β₂-adrenoceptors on the peripheral, circulating MNL were studied for comparison in the very same patient and at the same time.

Material and Methods

Patients

Eighteen patients were investigated who had to undergo lung-resection for bronchial carcinoma. Ten of these patients were serving as control subjects and did not take β-blockers, β₂-agonists, or other drugs interfering with the sympathetic nervous system. Beside this control group, eight patients revealed clinical signs of obstructive lung disease, and accordingly, PFT showed a decrease of peak expiratory flow and/or specific resistance. Thus, these patients were treated 24 to 72 hours preoperatively with the β₂-adrenoceptor agonist, terbutaline (0.5 mg subcutaneous injection, bid). The minimal cumulative terbutaline dose amounted to 1 mg. Two of
these treated patients already had been receiving long-term treatment (>6 months) with 10 mg terbutaline po. Further, three patients used β₂-inhalers with terbutaline sulphate (2 mg per day) additionally, and four patients received oral theophylline in a dose to up to 400 to 600 mg tid. In four of the eight treated patients, the PFT showed a specific airway resistance of more than 15 kPa; the other four patients suffered from a milder bronchial obstruction with a normal SRaw in accordance with their premedication.

The female to male ratio in the control group (mean age 63.6 ± 7.69 yr; range 52 to 77 yr) was 0.63, the terbutaline-treated patients (mean age 60.0 ± 3.05 yr; range 45 to 80 yr) revealed a f:m ratio of 0.14. There were five smokers and three nonsmokers (ratio 0.63) in the terbutaline-treated patients, vs two smokers and eight nonsmokers (ratio 0.2) in the untreated patients. All patients underwent anesthesia according to the same regimen (flunitrazepam, thiopental, pancuronium, enflurane), and informed consent was obtained for the procedure.

Human Lung Tissue

Approximately 10 to 20 g (wet weight) of peripheral, tumor-free lung tissue was obtained during thoracic surgery and suspended in ice cold TED-buffer (composition see below). The tissue pieces were immediately delivered to the laboratory and dissected free from pleura, connective tissue, visible bronchioli, and blood vessels. They were frozen in liquid nitrogen and stored at −80°C until used. Frozen tissue was allowed to thaw at 4°C within approximately two hours. In all subsequent steps, temperature was kept at 4 to 8°C. Storage did not alter β-adrenoceptor density. The tissue was minced with scissors, homogenized with an Ultra Turrax (Jahnke and Kunkel, Staufen, FRG) at 15,000 rpm two times at one minute and then once for two minutes at 1,500 rpm with an electric glass-fiber homogenizer (Jahnke and Kunkel, Staufen, FRG). The homogenate was filtered through a layer of gauze and centrifuged with a Beckman centrifuge I-128 (Beckman, Palo Alto, USA) for ten minutes at 500 g to remove undisrupted and fibrous tissue. Afterwards, the supernatant was recentrifuged at 100,000 g for 45 minutes. The pellet was resuspended in buffer I (composition see below), homogenized by hand with a glass-glass potter, and recentrifuged (100,000 g/45 minute). The final pellet was suspended in buffer I (10 vol) and the protein concentration determined according to Lowry et al.¹¹

Peripheral Mononuclear Leukocytes

During obliteration of the pulmonary vessel of this lung lobe which had to be resected, 30 ml of peripheral venous blood was drawn and anticoagulated with heparin. It was diluted 1:1 with PBS-buffer. For the MNL-preparation according to Böyum,¹⁸ samples of 20 ml were carefully layered on the Ficoll-Metrizoate solution. After centrifugation (Heraeus-Christ, Labofuge, FRG) at 1,000 g for 30 minutes the lymphocyte coat was washed two times (400 g/12 min with a Beckman LS-50 centrifuge) and finally resuspended in buffer II. The cells were counted (Hycel Counter HCZ22, Clinicon, Mannheim, FRG) and the total yield was in the range of 40 to 50 × 10⁶ cells. The cell suspension was adjusted to 3-4 × 10⁶ cells/ml as needed for binding experiments, frozen in liquid nitrogen, and stored at −80°C until use. No difference in receptor number or affinity was detected in frozen or intact cells (data not shown).

Radioligand-Binding Experiments

For binding experiments, the radiolabeled ligand (³²)iodocyanopindolol with a specific activity of 2,000 Ci/mol was used. Lung membrane studies were performed with 30 μg of protein/tube and in binding-experiments with MNL 0.5-1.0 × 10⁶ cells/tube were used. The final assay was performed in a total volume of 250 μl. The incubation was carried out at 37°C for 60 minutes. These conditions allowed a complete equilibration of the receptor with the radioligand. Determinations were performed in the presence and absence of 3 μmol/L (−)-propranolol at 10 to 400 pmol/L of 1-CYP. The reaction was terminated by rapid vacuum filtration through Whatman GF/F filters and washed with 10 ml of ice cold buffer I (lung tissue) or buffer II (MNL) three times. Radiocity was determined in a γ-scintillation counter (LKB Wallac, Freiburg, FRG). Specific binding was defined as the difference of binding in the absence and presence of 3 μmol/L (−)-propranolol. The maximal density (Bmax) and apparent affinity (Kd) of binding sites was obtained in individual experiments from Scatchard plots, determined by linear regression analysis. β-adrenoceptor subtypes were determined with competition experiments using the β₂-selective antagonist CGP 207.12 A and the β₁-selective antagonist ICI 118.551. The β₂ to β₁ ratio was calculated according to De Lean et al.³³

Substances and Buffers

Substances used were 1-CYP ((−)-3-(³²)-iodocyanopindolol) as the radiolabeled ligand (3000 Ci/mol). Buffer I contained (mmol/L) Tris 50; MgCl₂ 10; buffer II was buffer I plus sucrose 25 mmol/L. TED buffer consisted of (mmol/L) Tris 40; EDTA 23; thetreitol 1.

Figure 1. Competition of CGP 207.12 A (0.0001-100 μmol/l) and ICI 118.551 (0.0001-100 μmol/l) for 1-CYP binding to membranes of human lung tissue. The concentration of ³²I-CYP was 50 pmol/L. Each point represents the mean of triplicate observations. Ordinate: Percent of maximum specific binding with 50 pmol/L ³²I-CYP. Abscissa: Concentration of the β-adrenoceptor antagonist CGP 207.12 A and β₁-adrenoceptor antagonist ICI 118.551. Note that there is exclusively the β₂-adrenoceptor subtype in human peripheral lung.
Statistics

The data shown are means ± SEM. Statistical significance was estimated with Student's t-test for unpaired observations. A p-value of less than 0.05 was considered significant. K_d values and the drug concentration producing 50 percent of the maximal effect (EC50) were determined graphically in each individual experiment. The K_d and EC50 values are given as means with 95 percent confidence limits. Binding data of antagonist competition curves were analyzed by the computer modeling method of De Lean et al. One site and two site fits were tested for improvement of the fit by a F-test. The one or two site model was judged to be appropriate when it proved to be significantly better (p<0.0001) than the preceding one.

RESULTS

Human Lung Tissue

To determine the beta_1- and beta_2-adrenoceptor subpopulation, competition-binding experiments were performed using the selective beta_1-adrenoceptor antagonist CGP 207.12 A (0.0001-100 μmol/L) and the selective beta_2-adrenoceptor antagonist ICI 118.551 (0.0001-100 μmol/L). Typical competition experiments are shown in Figure 1. The competition curve of ICI 118.551 was biphasic. Analysis with the SCT-fit program according to De Lean et al revealed one predominant high affinity class of binding sites at the peripheral human lung, namely beta_2-adrenoceptors (>90 percent; mean K_a value: 6.8 nM). A beta_1-adrenoceptor subpopulation could not be detected (<10 percent). CGP 207.12 A revealed only one class of low affinity binding sites (ie, beta_2-adrenoceptors; mean K_a value: 4425 nM/L). Consistent with experiments with ICI 118.551, beta_1-adrenoceptors could not be measured.

Binding of I-CYP to membranes of lung parenchyma from ten untreated and eight terbutaline-treated patients with mild or moderate airway obstruction was performed and a typical saturation experiment of lung membranes from an untreated patient is shown in Figure 2. Saturation binding with I-CYP to lung membranes was monophasic. Equilibrium specific binding was saturable at concentrations between 200 and 400 pmol/L I-CYP. Linear transformation revealed a density of beta_2-adrenoceptors of 235 ± 26.2 fmol/mg protein in the untreated and 204 ± 19.5 fmol/mg protein in the treated patients [Fig 3]. The K_d was 50 pmol/L (44 to 58 pmol/L) in the untreated and 49 pmol/L (42 to 56 pmol/L) in the terbutaline-treated patients. The mean nonspecific binding amounted to 12.3 percent at 50 pmol/L I-CYP and to 28.3 percent at 400 pmol/L I-CYP. The Scatchard analysis showed a linear transformation of the saturation experiments. In the lung tissue of treated and untreated patients, there was no significant difference with regard to the total number of lung beta-adrenoceptors [Fig 3] and K_d values.

Human Peripheral Mononuclear Leukocytes

For subtype classification of beta-adrenoceptors, CGP 207.12 A (0.0001 to 100 μmol/L) and ICI 118.551 (0.0001-100 μmol/L) were used and revealed exclusively beta_2-adrenoceptors [Fig 4]. The mean K_a value was 3050 nM/L for CGP 207.12 A and 3.23 nM/L for ICI 118.551. I-CYP binding experiments with the MNL from the very same above-mentioned patients were performed under identical conditions as the experiments with lung membranes. Binding was saturable, monophasic and the plateau of the saturation curve was achieved at concentrations between 200 and 400 pmol/L I-CYP. A representative saturation experiment with MNL from an untreated patient is shown in Figure 5. In the group of the untreated patients, the linear transformation revealed a density...
FIGURE 3. Densities of β-adrenoceptors in human lung and mononuclear leukocytes of untreated and terbutaline-treated patients. Note that no correlation was obtained between receptor density in lung and MNL of untreated and treated patients. There was no change of β₂-adrenoceptor density in lung tissue of treated compared to untreated patients. In MNL, a significant decrease was detected.

of β₂-adrenoceptors (Bₘₐₓ) of 3.67 ± 0.69 fmol/10⁶ cells of MNL, whereas in the group of the terbutaline-treated patients, a Bₘₐₓ of 1.56 ± 0.16 fmol/10⁶ cells of MNL was found (p<0.05; Fig 3). The antagonist affinity (Kᵦ) amounted to 24 pmol/L (20 to 29 pmol/L) in the untreated and 28 pmol/L (20 to 39 pmol/L) in the treated patients (NS). The binding experiments revealed a mean nonspecific binding of 14.4 percent at 50 pmol/L I-CYP and 42.4 percent at 400 pmol/L I-CYP. Taken together, in human lung tissue and peripheral MNL, only β₂-adrenoceptors were detected. In lung tissue of patients with terbutaline treatment, no alteration in binding characteristics or total number of β-adrenoceptors occurred, whereas the Bₘₐₓ values obtained from MNL of treated patients were significantly diminished.

DISCUSSION

The use of terbutaline is well established in the treatment of increased airway resistance and acts as a β₂-adrenoceptor agonist.¹,² The efficacy is due to the stimulation of β₂-adrenoceptors in the lung, where the β₂-adrenoceptor subtype is predominant. This was initially demonstrated by Barnes et al.¹⁴,¹⁵ with autoradiographic examinations. In various systems, it is known that down-regulation of receptors occurs after continuous stimulation with agonists. This leads to a decrease in the response of the effector cells.⁷,¹⁶ Consequently, it was argued that in asthmatic and chronic obstructive pulmonary disease, treatment with β-adrenoceptor agonists would cause tachyphylaxis, followed by an impairment of airway response to β-sympathomimetic bronchodilators.⁸,¹⁷-⁹⁰ In another clinical study, Repsher et al.¹¹ found that this tachyphylaxis caused no significant clinical effect. Tachyphylaxis was only revealed in accordance with the duration of the bronchodilating action, but not to the peak expiratory flow. Other clinical studies evidence that long-term treatment with β₂-adrenoceptor

FIGURE 4. Competition of CGP 207.12 A (0.0001-100 μmol/L) and ICI 118.551 (0.0001-100 μmol/L) for I-CYP binding to human peripheral mononuclear leukocytes. The concentration of ¹²⁵I-CYP was 50 pmol/L. Each point represents the mean of triplicate observations. Ordinate: Percent of maximum specific binding with 50 pmol/L ¹²⁵I-CYP. Abscissa: Concentration of the β₂-adrenoceptor antagonist CGP 207.12 A and β₁-adrenoceptor antagonist ICI 118.551. Note that there is exclusively the β₂-adrenoceptor subtype in human MNL.

FIGURE 5. Specific binding of ¹²⁵I-CYP to peripheral mononuclear leukocytes of an untreated patient. Specific binding is defined as total minus nonspecific binding as measured in the absence and presence of 3 μmol/L (-)-propranolol and is expressed as fmol/10⁶ cells of MNL. Inset: ¹²⁵I-CYP bound per 10⁶ cells of MNL is plotted as a function of the ratio (B/F × 10⁻⁴) of bound ¹²⁵I-CYP to free ¹²⁵I-CYP. The intercept with the abscissa is the maximal number of binding sites (Bₘₐₓ), the slope is the apparent affinity (Kᵦ).
agonists in asthmatics is associated with a good bronchial dilatory response, even after repeated application of the compounds.22-24

For investigations of β-adrenoceptor function in human airways, lung material can rarely be obtained, whereas peripheral leukocytes are easily available and exclusively yield the β2-adrenoceptor subtype.25-27 Therefore, MNL had been claimed to reflect a reliable model for examining properties of β2-adrenoceptors in human lung tissue25,26 of healthy and asthmatic individuals.27,28 This conclusion was supported by results from animal models, which revealed changes in the density of β-adrenoceptors in rat lung and lymphocytes after β-antagonist treatment.29 Furthermore, radioligand-binding experiments with MNL from asthmatics, which were treated with β2-adrenoceptor agonists, revealed a down-regulation of β2-adrenoceptors,30,31 which had been interpreted as a defense mechanism of the cells to prevent excessive adenylyl cyclase stimulation.32 Down-regulation of β-adrenoceptors was observed as well in investigations of lung parenchyma from β-adrenoceptor agonist-treated guinea-pigs, however, at terbutaline doses highly above therapeutic levels (0.2 mg/kg tid).33 Former results from functional experiments on isolated trachea and lung parenchyma of guinea pigs also revealed the occurrence of tolerance to β-adrenoceptor agonist treatment.34,35,36 Hence, the question arises whether or not the lymphocyte is a suitable model for studying receptor regulation in human peripheral lung tissue as it was suggested from the data of the above-mentioned animal experiments.

With the highly selective β2-adrenoceptor antagonist ICI 118,551, both at MNL and lung membranes, exclusively adrenoceptors of the β2-subtype were found. The criteria of specific binding to β-adrenoceptors with saturability, reversibility, high affinity, rapid kinetics and competition for binding by agonists and antagonists were fulfilled. This indicates that both models were comparable by means of an identical type of receptor. Binding experiments with lung homogenates were also performed by Barnes et al.,37 using ([3H]-dihyroalpranolol as an unselective, radio-labeled β-adrenoceptor antagonist and butoxamine as the β2-selective antagonist. Accordingly, they also found that exclusively, the β2-adrenoceptor subtype occurs in the human lung. This is in concert with data from Davis et al38 who found that there is a 700-fold greater potency of propranolol compared with the β2-selective β-blocker practolol in inhibiting the cyclic AMP response to isoprenaline in homogenates of human lung, suggesting that the β2-adrenoceptor predominates. Hence, the data of the present study show that in both human lung and MNL, the same β2-adrenoceptor subtype occurs.

In the lymphocytes from treated vs untreated patients, the number of the β2-adrenoceptors revealed a significant down-regulation to 43 percent. One might argue that the operative procedure might have influenced these results. However, anesthesia and other operative procedures had been identical in all the patients. Thus, individual effects which could alternate the β-adrenoceptor regulation by an increased catecholamine secretion during the operation are unlikely to explain the difference between the studied groups. Similar data were found by several other investigators after treatment of healthy adults and asthmatics with terbutaline.1,10,39,40 The down-regulation of adrenoceptors of MNL in each treated patient indicates that sufficient blood levels of terbutaline for adrenoceptor regulation had been achieved. This was additionally supported by a good clinical bronchodilatory effect of the medication, even in the two patients who had been on a long-term treatment with β-sympathomimetics. Moreover, studies of Leferink et al41 indicate that after subcutaneous administration of 0.5 mg terbutaline, higher and more constant therapeutic blood levels for bronchodilatation can be achieved compared to the oral form of application. In contrast to the results in MNL of pretreated patients, no down-regulation occurred in the total amount of parenchymal β-adrenoceptors in the lung membranes of these very same patients. Moreover, the results of this study show that no correlation exists between the β2-adrenoceptor density obtained in MNL and in the different cell types of lung membranes. The experiments provide evidence that lymphocytes are more susceptible to β-adrenoceptor down-regulation than is human peripheral lung tissue. Previously, experimental evidence was raised by Hasegawa and Townley,32 who found a greater sensitivity of β-adrenoceptor down-regulation by agonists in splenic lymphocytes of rats than in lung tissue. Hitherto, this study presents the first investigations in which β2-adrenoceptors of human lung and MNL were compared in the same patients at the same time, with and without β2-adrenoceptor agonist therapy.

In conclusion, β2-adrenoceptor treatment is effective in down-regulating β2-adrenoceptors of human peripheral MNL. However, these alterations are not obtained in human lung parenchyma. Conclusions concerning conditions of β-adrenoceptors of lung parenchyma should, therefore, not be drawn from results obtained with MNL. The resistance of lung β2-adrenoceptors to application of therapeutic doses of terbutaline provides an explanation for the continuous beneficial effect of β2-sympathomimetic bronchodilators in the treatment of obstructive lung disease.

REFERENCES

1 Williams SJ, Wimmer SJ, Clark TJH. Comparisons of inhaled and intravenous terbutaline in acute severe asthma. Thorax
Terbutaline-induced AE


Galant SP, Underwood S, Duriseti L, Insel PA. Characterization of high-affinity beta,-adrenergic receptor binding of (+)-'125I-dihydroalprenolol to human polymorphonuclear cell particulates. J Lab Clin Med 1978; 92:613-18


Leferric JG. Terbutaline: analytical, clinical pharmacological and toxicological aspects. Ammerlaan BV. Wateringen: 1979; 1-134


Conolly ME, Davies DS, Dollery CT, George CF. Resistance to beta-adrenoceptor stimulants (a possible explanation for the rise in asthma deaths). Br J Pharmac 1971; 43:399-402


Aarons RD, Molinoff PB. Changes in the density of beta adrenergic receptors in rat lymphocytes, heart and lung after chronic treatment with propranolol. J Pharmacol Exp Ther 1982; 221:439-43


Benoit CJ, El-Fellah MS, Schneider R, Wade OL. Tolerance to sympathomimetic bronchodilators in guinea-pig isolated lungs following chronic administration in vivo. Br J Pharmacol 1975; 55:547-54

