Pharmacological actions of the selective and non-selective β -adrenoceptor antagonists celiprolol, bisoprolol and propranolol on human bronchi

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- 1 The pharmacological actions of the β -adrenoceptor antagonists, celiprolol, bisoprolol and propranolol were investigated in human lung tissue by radioligand binding experiments as well as in human isolated bronchi by functional experiments in organ baths.
- 2 Data from lung tissue were compared to those obtained from myocardial membranes.
- 3 Lung tissue was obtained from 10 patients having undergone lung resection for bronchial carcinoma and myocardial tissue from a patient who had received a heart transplantation.
- 4 In radioligand binding experiments, celiprolol exhibited a high affinity binding to β_1 -adrenoceptors in heart and a low affinity binding to β_2 -adrenoceptors in lung tissue. The selectivity obtained for the β_1 -adrenoceptor was calculated to a factor of eleven.
- 5 Compared to bisoprolol and propranolol, celiprolol elicited the lowest affinity for the β -adrenoceptor, as judged from the K_i -values.
- 6 In the absence and presence of the guanine nucleotide Gpp(NH)p celiprolol did not affect receptor binding.
- 7 In functional experiments on intact bronchi, celiprolol, bisoprolol and propranolol failed to produce relaxation ($\pm$ forskolin) or a significant difference in efficacy in antagonizing the relaxant effects of isoprenaline. However, a rank order of potencies was revealed (propranolol:bisoprolol:celiprolol = 46:12:1).
- 8 Plasma concentrations for celiprolol and bisoprolol usually achieved *in vivo* were below the IC_{50} value obtained *in vitro*. In contrast, for propranolol, plasma concentrations were nearly identical with the IC_{50} value.
- 9 It is concluded that celiprolol is a selective β_1 -adrenoceptor antagonist on human heart and has no agonistic properties on intact human bronchi. Compounds such as celiprolol and bisoprolol may in comparison to propranolol, possess reasonable therapeutic advantages in the treatment of patients with obstructive lung disease due to their low affinity for β_2 -adrenoceptors.

Keywords: Human bronchi; human lung; β -adrenoceptor antagonists; cardioselectivity; intrinsic sympathomimetic activity; celiprolol; bisoprolol; propranolol; forskolin; asthma

Introduction

β_2 -Adrenoceptor antagonists are potentially harmful to patients with airway obstruction and chronic bronchitis (Tattersfield, 1986; Dorow, 1988a); therefore, β -adrenoceptor antagonists should be avoided in these patients (Dorow, 1988b). However, it has been suggested that some newer β -adrenoceptor antagonists, e.g. celiprolol, possess certain properties, such as cardioselectivity and intrinsic sympathomimetic activity or both, which could make them potentially suitable for patients with obstructive lung disease. Clinical studies have shown that celiprolol can elicit some bronchodilator effects (Doshan *et al.*, 1986b; Pruss *et al.*, 1986). Although it has been suggested that β_2 -adrenoceptor agonist properties may play a role in this context, pharmacological characterization of the compounds using human tissue and based upon rigorous criteria, i.e. measurement of the affinity of binding to β_2 -adrenoceptors, potency and efficacy in antagonizing the isoprenaline effect, investigation of partial agonist and relaxant activity, have not been carried out. Therefore, the pharmacological properties of celiprolol were studied in human lung tissue using radioligand binding experiments. The data obtained were subsequently compared to those collected from human cardiac membranes. To deter-

mine whether the biochemical results are relevant for the bronchoconstrictor and bronchodilator responses of bronchial smooth muscles, celiprolol, a β_1 -selective adrenoceptor antagonist with intrinsic sympathomimetic activity (ISA), bisoprolol a β_1 -selective adrenoceptor antagonist without ISA, and propranolol a non-selective β -adrenoceptor antagonist were investigated in human isolated bronchial rings.

Methods

Patients

Informed consent was obtained from a total of 10 patients chosen for this study (2 females, 8 males) requiring pneumonectomy or lobectomy for the treatment of bronchial carcinoma (Table 1). All of the patients were subjected to preoperative lung function testing whereby airway obstruction was excluded. Every one of the ten patients were current smokers with an average intake of 10–20 cigarettes per day for over a period of at least 15 years. Age ranged between 34 and 83 (mean $\pm$ s.e.mean: 59.2 ± 4.4). None of the patients was taking any medication which interfered with the sympathetic or parasympathetic nervous system. All were administered anaesthesia according to the same regimen: Flunitrazepam, thiopental, pancuronium and isoflurane.

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Table 1 Biographical and lung function data of the patients

Initials	Sex	Age	FEV ₁ (%VC)	Resistance (kPa(1 s ⁻¹) ⁻¹)
S.W.	m	83	64	0.31
H.O.	m	57	68	0.23
H.A.	m	71	72	0.27
R.M.	m	64	72	0.23
F.K.	m	69	65	0.22
S.A.	f	34	58	0.27
M.D.	m	50	77	0.17
K.W.	f	45	90	0.27
S.G.	m	63	75	0.19
D.R.	m	56	72	0.18
Mean ± s.e.mean		59.2 4.4	71.3 2.74	0.23 0.01

FEV₁ = forced expiratory volume in 1 s.
VC = vital capacity.

Human lung tissue

Approximately 100 g (wet weight) tumour-free lung parenchyma was obtained during thoracic surgery and suspended in preaerated ice cold Tyrode-solution (composition see below) as described recently (Hauck *et al.*, 1990; Böhm *et al.*, 1991). The hypoxic delay was kept to a minimum, usually less than 1 min. After rapid transportation to the laboratory, bronchi with an inner diameter between 4–6 mm were prepared from the resected lung tissue. Approximately 10 g (wet weight) of lung tissues were frozen in liquid nitrogen and stored at –80°C until use. For radioligand binding experiments, frozen tissue was allowed to thaw to 4°C within approximately 2 h. In all subsequent steps, temperature was kept at 4–8°C. Storage did not alter β -adrenoceptor density. The tissue was minced with scissors, homogenized twice for 1 min with an Ultra Turrax (Jahnke & Kunkel, Staufen, Germany) at 15,000 r.p.m. and then once for 2 min at 1500 r.p.m. with an electric glass-Teflon homogenizer (Jahnke & Kunkel, Staufen, Germany). The homogenate was filtered through a layer of gauze and centrifuged in a Beckman centrifuge I-218 (Beckman, Palo Alto, USA) for 10 min to remove undisrupted and fibrous tissue. The supernatant was then recentrifuged at 100,000 *g* for 45 min. The pellet was resuspended in incubation buffer (composition see below), homogenized by hand with a glass-glass homogenizer and recentrifuged (100,000 *g*/45 min). The final pellet was suspended in 10 volumes of the incubation buffer and the protein concentration determined according to Lowry *et al.* (1951).

Human myocardium

Myocardium was obtained from a 60 year old male patient with terminal heart failure due to dilated cardiomyopathy who had undergone heart transplant surgery. The tissue was chilled in 30 ml of ice-cold homogenization buffer (Tris HCl 10 mmol l⁻¹, EDTA 1 mmol l⁻¹ pH 7.4) and was disrupted with an Ultra Turrax agitator (see above). The homogenate was spun at 2,000 *g* for 10 min. The supernatant was retained and the pellet was discarded. This homogenate was diluted with an equal volume of ice-cold KCl 1 mol l⁻¹ and stored on ice for 10 min. The supernatant was centrifuged at 100,000 *g* for 45 min. The pellet was resuspended in 50 volumes of incubation buffer, homogenized for 1 min in a glass-glass flask and recentrifuged at 100,000 *g* for 45 min. The final pellet was resuspended in incubation buffer (50 vol). The homogenate was then stored as previously described (Böhm *et al.*, 1992). Radioligand binding experiments were subsequently performed in accordance to the undermentioned experiments with lung parenchyma.

Radioligand binding experiments

In order to quantify the affinity constants and potential partial agonist properties of β -adrenoceptor antagonists at β_1 - and β_2 -adrenoceptors, competitive experiments for binding of [¹²⁵I]-iodocyanopindolol (specific activity 2000 Ci mmol⁻¹) to β -adrenoceptors were performed. Specific binding was defined as the difference in binding in the absence and in the presence of propranolol (3 μ mol l⁻¹). Propranolol, celiprolol and bisoprolol at increasing concentrations (0.0001–100 μ mol l⁻¹) were used for binding experiments with lung, and celiprolol with myocardial tissue. The experiments were performed in the presence and absence of Gpp(NH)p to evaluate agonist properties of the β -adrenoceptor antagonists by changing the agonist affinity state of the binding site in the presence of the metabolically stable guanine nucleotide. Usually, the non-hydrolysable guanine-nucleotide leads to a reduction in binding that results in a steeper and rightward shift of the competitive curve if there is agonist activity of the compound studied at the β -adrenoceptors (Mattera *et al.*, 1985; Jasper *et al.*, 1988; Jasper & Insel, 1992). Lung membrane studies were performed with 30 μ g of protein/tube. Each result represents four experiments in triplicate determinations. To block potentially existing β_2 -adrenoceptors in the myocardial membranes, the β_2 -adrenoceptor antagonist ICI 118,551 (50 nmol l⁻¹) was present in the reaction medium. To block potentially existing β_1 -adrenoceptors in the lung membranes, CGP 20,712A (300 nmol l⁻¹) was added to the reaction medium. The binding data of celiprolol obtained in human lung tissue were compared to those from the experiments in human myocardium.

Human isolated bronchi

For the functional investigations, bronchi were cut into rings, 2–3 mm thick and stored overnight in Tyrode solution at 4°C containing tobramycin (5 mg l⁻¹) and penicillin (0.03 mg l⁻¹; de Jongste *et al.*, 1985). The solution was aerated with a slow continuous flow of 95% O₂ and 5% CO₂. This step assured a complete washout of blood compounds and narcotics. After 12 h the bronchial rings were suspended in 75 ml organ chambers, filled with preaerated Tyrode solution at pH 7.4 and maintained at 37°C. Isometric tension was measured with an inductive Fleck force transducer attached to a Hellige Helco Scriptor recorder. Following an equilibration period of 90 min, during which the bathing solution was changed at 30 min intervals, the bronchial rings were mounted under an isometric load of 2 g. Cumulative concentration-response curves were subsequently constructed. All drugs were freshly prepared immediately prior to the experiment and maintained on ice until use. For construction of concentration-response curves, the compounds were added in a cumulative manner. For contraction experiments, the concentrations of the different β -adrenoceptor antagonists ranged from 0.01 μ mol l⁻¹ to 100 μ mol l⁻¹, and for relaxation experiments from 0.001 μ mol l⁻¹ to 1000 μ mol l⁻¹. In relaxation experiments, bronchial rings were first precontracted with carbachol at 0.4 μ mol l⁻¹ (EC₅₀). This concentration has been shown in former experiments to evoke the half maximal contraction (not shown). In the experiments designed to evaluate the contractile response of the β -adrenoceptor antagonists, bronchial rings were first relaxed with isoprenaline 0.3 μ mol l⁻¹, a concentration which results in the half maximal relaxation (IC₅₀) with this substance. In order to unmask the agonist properties of the β -adrenoceptor antagonists via facilitation of β -adrenoceptor-G-protein coupling, relaxation experiments were repeated using the diterpene derivative, forskolin at a concentration of 0.01 μ mol l⁻¹.

Substances and buffers

[¹²⁵I]-CYP ((–)-3-[¹²⁵I]-iodocyanopindolol) as the radiolabelled ligand (specific activity 2000 Ci mmol⁻¹) was obtained

from Amersham-Buchler (Braunschweig, Germany) and the selective β_1 -adrenoceptor antagonist CGP 20,712A [2-hydroxy-5-[2-(hydroxy-3-(4-[(1-methyl-4-trifluoromethyl)-1-H-imidazol-2-yl]-phenoxy) propyl) aminoethoxy] benzamide] from Ciba-Geigy AG (Basel, Switzerland). The selective β_2 -adrenoceptor antagonist ICI 118,551 [erythro-($\pm$)-1-(7-methylindian-4-yloxy)-3-isopropyl-aminobutan-2-ol-hydrochloride] was obtained from Zeneca Pharmaceuticals (Macclesfield, U.K.). Celiprolol was from Upjohn, ($\pm$)-isoprenaline HCl from Boehringer Ingelheim (Ingelheim, Germany). Forskolin was donated by Dr Metzger, Hoechst AG (Frankfurt, Germany). The Tyrode solution consisted of (mmol l⁻¹): NaCl 119.8, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1.05, NaH₂PO₄ 0.42, NaHCO₃ 22.6, Na₂EDTA 0.05, ascorbic acid 0.28 and glucose 5.0. The incubation buffer contained (mmol l⁻¹) Tris 50, MgCl₂ 10.

Statistics

In functional studies EC₅₀ and IC₅₀ values were estimated graphically as the concentrations producing half maximal tension or half maximal relaxation attained with bronchi. Data shown for contraction experiments are mean $\pm$ s.e. mean. Statistical significance was estimated with Student's *t* test for unpaired observations. A *P*-value of less than 0.05 was considered to be significant. *K*₁-values in competition experiments were calculated according to Cheng & Prusoff (1973). Agonist competitive curves were analysed with the SCTFIT-programme (de Lean *et al.*, 1982). Curve fits assuming one, two and three affinity states were compared with an *F*-test. The significantly (*P* < 0.005) best fit was accepted.

Results

Radioligand binding experiments

With celiprolol, a monophasic antagonism to [¹²⁵I]-CYP binding was achieved in human lung and myocardial membranes. This effect occurred with a high affinity for β_1 -adrenoceptors in heart and with low affinity for β_2 -adrenoceptors in lung membranes (Table 2).

Competition experiments have shown that celiprolol exhibits, neither at lung β_2 -adrenoceptors nor at heart β_1 -adrenoceptors, partial agonistic activity. From the *K*₁ values, a β_1 -adrenoceptor selectivity of celiprolol of a factor of eight (in the absence of Gpp(NH)p) or eleven (in the presence of Gpp(NH)p) was determined (Table 2).

Next the full β -adrenoceptor agonist, isoprenaline, was studied for comparison, as to whether guanine-nucleotide-sensitive high and low affinity binding of agonists could be detected within lung or cardiac membranes (Figure 1). Isoprenaline showed biphasic competition for lung β_2 -adrenoceptors. The concentration-binding curve was shifted to the right by Gpp(NH)p, resulting in a fit to one low affinity

binding site. This demonstrates that the technique used was sensitive to separate high and low affinity states for agonist binding.

The same experiments were also performed with propranolol and bisoprolol in comparison to celiprolol. As summarized in Figure 2, none of the three β -adrenoceptor antagonists elicited agonist characteristics. Table 2 sum-

Figure 1 Inhibition of [¹²⁵I]-iodocyanopindolol ([¹²⁵I]-CYP) binding to lung β_2 -adrenoceptors by isoprenaline in the absence (□) and presence (■) of Gpp(NH)p (100 μ mol l⁻¹). Ordinate scale: specific binding as percentage of [¹²⁵I]-CYP specifically bound. Abscissa scale: concentration of isoprenaline in μ mol l⁻¹. Note that with Gpp(NH)p a low affinity binding site was observed.

Figure 2 Inhibition of [¹²⁵I]-iodocyanopindolol ([¹²⁵I]-CYP) binding to lung β_2 -adrenoceptors by isoprenaline ($\blacktriangle$, $\triangle$), bisoprolol ($\bullet$, $\circ$) and celiprolol ($\blacksquare$, $\square$) in the absence (open symbols) and presence (solid symbols) of Gpp(NH)p (100 μ mol l⁻¹). Ordinate scale: specific binding as percentage of [¹²⁵I]-CYP specifically bound. Abscissa scale: concentrations of the different β -adrenoceptor antagonists. Note that propranolol demonstrated the highest affinity for the β_2 -adrenoceptors, followed by bisoprolol and celiprolol. No influence was observed of the presence of Gpp(NH)p.

Table 2 Equilibrium dissociation constants *K*₁ for competing β -adrenoceptor antagonists in membranes from human peripheral lung and human heart

	- Gpp(NH)p (μ mol l ⁻¹)	+ Gpp(NH)p (μ mol l ⁻¹)
<i>Human lung</i> (+ CGP 207.12A)		
Propranolol	0.0006 (0.00045–0.0009)	0.00075 (0.00036–0.0016)
Bisoprolol	0.4 (0.25–0.65)	0.45 (0.25–0.85)
Celiprolol	2.8 (1.35–5.85)	3.55 (1.05–10.7)
<i>Human heart</i> (+ ICI 118.551)		
Celiprolol	0.35 (0.28–0.4)	0.31 (0.23–0.43)

*K*₁ values are given with 95% confidence limits.

marizes the K_i -values of the β -adrenoceptor antagonists with and without Gpp(NH)p. With the β -adrenoceptor antagonists, a difference in the K_i -values within the 95% confidence limits was measured in the presence and absence of $100 \mu\text{mol l}^{-1}$ Gpp(NH)p (Table 2, Figure 2). Comparison of the compounds studied showed that the β -adrenoceptor affinity of propranolol was significantly higher (K_i : $0.0006 \mu\text{mol l}^{-1}$), than that of bisoprolol (K_i : $0.4 \mu\text{mol l}^{-1}$) and celiprolol (K_i : $2.8 \mu\text{mol l}^{-1}$). Therefore, the rank order of affinity is propranolol > bisoprolol > celiprolol resulting in relative affinities of 4667: 7: 1.

Functional studies

In order to study the bronchoconstrictor and bronchodilator effects of the β -adrenoceptor antagonists, functional experiments with bronchial rings were performed. In Figure 3, original recordings illustrate the effect of isoprenaline and celiprolol in human bronchial rings. At $0.4 \mu\text{mol l}^{-1}$ carbachol (i.e. the concentration producing half maximal effect on tension development) the evoked tension increased to 9.6 mN. Isoprenaline, added at a half maximal concentration, led to relaxation below the resting tension. After equilibration under these conditions, celiprolol produced an increase in tension which was maximal at a concentration of $100 \mu\text{mol l}^{-1}$. The maximal relaxant effect of papaverine at $100 \mu\text{mol l}^{-1}$ is also illustrated.

In order to evaluate efficacy and potency of the potential bronchoconstrictor action, bronchi were concentration-dependently contracted with the β -adrenoceptor antagonists. A similar efficacy (judged from the maximal effects) of propranolol, bisoprolol and celiprolol in evoking contraction in the human bronchi was observed (not shown).

In order to evaluate the potency of these compounds, the IC_{50} -values (cf. Table 3) were calculated from concentration-response curves (Figure 4). The half maximal effects of propranolol occurred at $0.1 \mu\text{mol l}^{-1}$, for bisoprolol at $1.2 \mu\text{mol l}^{-1}$ and for celiprolol at $4.6 \mu\text{mol l}^{-1}$ (Table 3). Thus, the rank order of potency is propranolol > bisoprolol > celiprolol, resulting in relative potencies of 46: 12: 1.

In order to evaluate the potential clinical relevance of the bronchoconstrictor response, the IC_{50} values *in vitro* were compared with the therapeutic drug concentrations *in vivo*. As shown in Table 3, the reported plasma concentrations of propranolol subsequent to an oral dose of 80 mg, were within the range which evoked the half maximal bronchial constriction (IC_{50}) *in vitro*. In contrast, the plasma concentrations following an oral application of 10 mg bisoprolol, and 200 mg celiprolol, respectively, were below the range which led to a half maximal contraction at bronchial rings *in vitro*. The therapeutic range of 80 mg propranolol (plasma concen-

tration: $0.289 \pm 0.006 \mu\text{mol l}^{-1}$, Naline *et al.*, 1990) inhibited the isoprenaline effect on human bronchi by about 58%; 10 mg bisoprolol (plasma concentration: $0.15 \pm 0.006 \mu\text{mol l}^{-1}$; Naline *et al.*, 1990) antagonized the isoprenaline effect by about 5% and 200 mg celiprolol (plasma concentration: $1.35 \pm 0.56 \mu\text{mol l}^{-1}$; Schmidt *et al.*, 1985) diminished the isoprenaline effect by around 20% (Figure 4).

Further experiments were designed to determine whether the compounds were capable of producing direct broncho-relaxant effects. As shown in Figure 5 (concentration-response curves), with isoprenaline full relaxation of the

Figure 3 Original recording which illustrates the effect of celiprolol on tension development in human bronchial rings. Note that celiprolol maximally antagonizes the isoprenaline effect at $100 \mu\text{mol l}^{-1}$. This was completely reversed by papaverine, $100 \mu\text{mol l}^{-1}$.

Figure 4 Concentration-response curves for the effect on force of contraction of propranolol (▲), bisoprolol (●) and celiprolol (■) on carbachol ($0.4 \mu\text{mol l}^{-1}$) precontracted and isoprenaline ($0.3 \mu\text{mol l}^{-1}$) relaxed human bronchi. Ordinate scale: increase in tension development as percentage of the maximal effect. Abscissa scale: concentration of the different β -adrenoceptor antagonists. Note the rank order of potency was: propranolol > bisoprolol > celiprolol.

Table 3 IC_{50} and EC_{50} concentrations of agonists and antagonists in human bronchi calculated from functional investigations *in vitro*

Substance	n	IC_{50} EC_{50} ($\mu\text{mol l}^{-1}$)	Maximal effect (mN)	Plasma level ($\mu\text{mol l}^{-1}$)
Isoprenaline ¹	7	0.3 (0.2–0.4)	–	–
Isoprenaline ²	5	0.016 (0.007–0.04)	–	–
Propranolol ³	4	0.1 (0.074–0.3)	9.15 ± 2.2	0.289 ± 0.006^4
Bisoprolol ³	6	1.2 (0.7–2.1)	9.54 ± 3.5	0.15 ± 0.006^5
Celiprolol ³	5	4.6 (3.1–6.8)	7.78 ± 0.5	1.35 ± 0.56^6

Values are mean with 95% confidence limits. Plasma levels *in vivo* of the investigated compounds are shown for comparison. Values are mean $\pm$ s.e.mean.

¹Precontraction with EC_{50} carbachol; ²precontraction with EC_{50} carbachol and EC_{20} forskolin; ³after precontraction with EC_{50} carbachol and EC_{50} isoprenaline relaxation.

⁴After 80 mg propranolol p.o. (Naline *et al.*, 1990); ⁵After 10 mg bisoprolol p.o. (Naline *et al.*, 1990); ⁶After 200 mg celiprolol p.o. (Schmidt *et al.*, 1985).

Figure 5 Concentration-response curves for the effect of isoprenaline in the absence ($\square$) and presence ($\blacksquare$) of forskolin ($0.01 \mu\text{mol l}^{-1}$) on carbachol ($0.4 \mu\text{mol l}^{-1}$)-precontracted bronchi. Ordinate scale: tension of bronchi as percentage of the maximal relaxant effect of papaverine. Abscissa scale: concentration of isoprenaline. Note that forskolin led to an increase in potency of isoprenaline in relaxing human bronchi by a factor of 18.

precontracted bronchi could be achieved. The threshold concentration for relaxation was $0.01 \mu\text{mol l}^{-1}$ and the maximum relaxation was achieved at $10 \mu\text{mol l}^{-1}$ without an additional effect of papaverine. After forskolin ($0.01 \mu\text{mol l}^{-1}$) was added, the half maximal relaxation occurred at lower concentrations, i.e. 0.016 (0.007 – 0.04) $\mu\text{mol l}^{-1}$, compared to 0.3 (0.2 – 0.4) $\mu\text{mol l}^{-1}$ in the absence of forskolin. This indicated an increase in potency evoked by forskolin by a factor 18.7. Since forskolin facilitates the coupling of β -adrenoceptors to adenylate cyclase (Böhm *et al.*, 1992), experiments with celiprolol were performed in the absence and presence of forskolin in order to sensitize the β -adrenoceptor-G-protein system towards stimulation by partial agonists. Celiprolol did not elicit a significant bronchorelaxant response at concentrations below $100 \mu\text{mol l}^{-1}$. The same experiments with forskolin did not lead to an increase in potency of the relaxant activity of celiprolol which occurred only at concentrations higher than $100 \mu\text{mol l}^{-1}$.

Discussion

In the present study, the pharmacological properties of celiprolol, bisoprolol and propranolol on human lung tissue as well as human bronchi were investigated. Because of its high affinity for β_1 -adrenoceptors (van Inwegen *et al.*, 1984), often termed cardioselectivity, celiprolol is frequently used in the treatment of such cardiovascular diseases as coronary heart disease and hypertension (Prichard & Tomlinson, 1986; Clauzel *et al.*, 1988). Since in the human lung, the β_2 -adrenoceptor subtype predominates (Barnes *et al.*, 1983; Carstairs *et al.*, 1985), it has been suggested that in the treatment of patients with obstructive airway disease the β_1 -adrenoceptor selectivity of celiprolol, together with its supposed partial agonist activity on β_2 -adrenoceptors, could provide some advantages by evoking bronchodilator effects (Doshan *et al.*, 1986b; Matthys *et al.*, 1986).

The results of this study show that within myocardial membranes, celiprolol exhibited high affinity antagonist binding to β_1 -adrenoceptors. Its binding to β_2 -adrenoceptors was studied in lung tissue with radioligand binding experiments performed on membranes from lung tissue. It could be demonstrated that for celiprolol, there is only one low affinity binding site of the β_2 -adrenoceptor subtype. The use of Gpp(NH)p provided no evidence for agonist properties.

Since bisoprolol should exhibit a similar β_1 -adrenoceptor

selectivity (Schliep & Frey, 1984), a comparative investigation of these compounds and the non-selective β -adrenoceptor antagonist, propranolol was carried out. Celiprolol demonstrated the lowest β_2 -adrenoceptor affinity as judged from the K_i -values, followed by bisoprolol and propranolol. These data reveal celiprolol to be a highly selective compound at β_1 -adrenoceptors but without β -adrenoceptor agonist properties at the β_2 -adrenoceptor subtype.

Dhein *et al.* (1992) reported a β_2 -adrenoceptor-mediated sympathomimetic activity of celiprolol, as shown by its vasodilator effect. These findings raised the question as to whether these agonist effects could also be demonstrated to activate β_2 -adrenoceptors within the human lung. Such effects could possibly provide support for cyclic AMP formation in bronchial smooth muscles, with initiation of bronchodilator effects (Jasper & Insel, 1992). In competition radioligand binding experiments with $100 \mu\text{mol l}^{-1}$ of the non-hydrolysable guanine-nucleotide Gpp(NH)p, no alteration in the affinity state of celiprolol binding was measured (Mattera *et al.*, 1985; Jasper *et al.*, 1988). Hence, no evidence for partial agonist activity was detected. In this respect, the effect of celiprolol did not differ from the effect of the other β -adrenoceptor antagonists. In contrast, guanine-nucleotide-sensitive high- and low-affinity binding was observed with the full β -adrenoceptor agonist, isoprenaline. Thus, with this technique, celiprolol was classified as an antagonist at β_2 -adrenoceptors of peripheral lung tissue. Because at this stage the question remained open as to whether the pharmacological properties (e.g. β -adrenoceptor-G-protein coupling) could be different in intact human bronchi, the effects of celiprolol on tension development were studied. In contrast to the β -adrenoceptor agonist, isoprenaline, celiprolol failed to relax isolated intact human bronchi *in vitro*.

Similar results were obtained in the presence of the diterpene derivative forskolin (Seamon & Daly, 1989), which has been reported to unmask increases in cyclic AMP-formation by partial β -adrenoceptor agonists in human lymphocytes via facilitation of β -adrenoceptor-G-protein coupling (Jasper *et al.*, 1989). Even under these conditions, celiprolol did not evoke bronchodilator effects, which would have served as evidence of a β -adrenoceptor agonist action.

In the case of celiprolol, clinical studies (Rosenthal *et al.*, 1985; Schindl *et al.*, 1986; van Zyl *et al.*, 1989) have shown that during treatment, the FEV₁ will not be adversely affected in patients with obstructive lung disease. In addition, it has been demonstrated that after application of celiprolol no decline in the bronchodilator activity of β_2 -sympathomimetic drugs occurs. In a study (Carnathan *et al.*, 1988) on cats infused with 5-hydroxytryptamine it was shown that after infusion of celiprolol ($35 \mu\text{g kg}^{-1} \text{min}^{-1}$), a reduction of airway resistance was induced. In contrast, clinical studies (Matthys *et al.*, 1986) indicate that after pretreatment with propranolol a marked antagonism to the bronchodilator response of a β_2 -adrenoceptor agonist treatment was observed.

Thus, there is an obvious discrepancy between the beneficial findings concerning the bronchodilator effects of celiprolol in patients *in vivo* (Rosenthal *et al.*, 1985; Matthys *et al.*, 1986; Schindl *et al.*, 1986; van Zyl *et al.*, 1989) and the lack of β_2 -adrenoceptor agonist activities of the compound *in vitro* (this study). The tone of the bronchial smooth muscles is under constant regulatory control of the sympathetic nervous system (Tattersfield, 1986). Therefore, it is conceivable that a portion of the β -adrenoceptors are continuously stimulated by endogenous catecholamines (Barnes *et al.*, 1981; Ind *et al.*, 1985; Tattersfield, 1986). From this point of view, an alternative explanation for the beneficial effect of celiprolol (with low incidence and severity of adverse reactions in patients) would be a low antagonist affinity for β_2 -adrenoceptors. This low affinity was established in our radioligand binding experiments, in comparison with the other compounds. The pharmacological properties of celiprolol would result only in a small antagonism to the stimulation of β_2 -adrenoceptors by endogenous catechola-

mines resulting in less adverse effects on bronchial tone. Since attacks of asthma, associated with dyspnoea and tachycardia, are presumably accompanied by sympathetic activation, this mechanism might become increasingly relevant in severe cases of this disease.

To examine this latter hypothesis experimentally, the situation was mimicked *in vitro* by testing the effects of celiprolol and the other β -adrenoceptor antagonists in the presence of an isoprenaline-induced half maximal bronchodilator response. All the antagonists produced complete antagonism of the bronchial relaxant response to isoprenaline. However, it is worth noting that the drug concentrations necessary to evoke the maximal and half-maximal bronchoconstrictor effects were quite different. This difference in potency amounted for propranolol to a factor 12 and 46 respectively, in comparison to bisoprolol and celiprolol, i.e. propranolol antagonized the bronchorelaxant effects of isoprenaline at the lowest concentration (IC_{50} : $0.1 \mu\text{mol l}^{-1}$).

It remained unclear as to whether the latter findings could be influenced by non-specific effects of the β -adrenoceptor antagonists. However, a close correspondence between the affinity of the compounds for the β -adrenoceptor obtained in radioligand binding studies and the IC_{50} -values for their bronchoconstrictor actions was observed. Thus, the results presented favour the suggestion that the different effects of the β -adrenoceptor antagonists on intact preparations are based upon their individual pharmacological profiles of interaction with the β -adrenoceptor.

In order to evaluate further the clinical relevance of these data, the plasma concentrations resulting from oral doses of propranolol, 80 mg (Naline *et al.*, 1990), bisoprolol, 10 mg (Naline *et al.*, 1990) and celiprolol, 200 mg (Schmidt *et al.*, 1985) were compared with the functional effects which had been evoked at those concentrations on intact preparations of human bronchi *in vitro*. This approach allowed the detection of an apparent difference between the β_1 -selective and the non-selective β -adrenoceptor antagonists. It was to be expected that celiprolol and bisoprolol would exert smaller bronchoconstriction at therapeutic drug levels than does propranolol. Propranolol evokes half maximal contraction in

human bronchi at therapeutic concentrations, whereas bisoprolol, at therapeutic concentrations, antagonizes the isoprenaline relaxed bronchi only by some 5% and celiprolol by just 20%. These *in vitro* data explain the results of clinical studies in which celiprolol was found to be at a lesser disadvantage in the adverse influencing of bronchial resistance. For propranolol, bronchoconstrictor action is reported at oral doses of 40, 100 and 200 mg *in vivo* (Doshan *et al.*, 1986a; Schindl *et al.*, 1986; Bruschi *et al.*, 1988).

It is concluded that celiprolol has a relevant β_1 -adrenoceptor selectivity which amounts to a factor of 8–11 in comparison to β_2 -adrenoceptors. Agonist properties could not be demonstrated on intact human bronchi or lung membranes even though the β -adrenoceptor-G-protein coupling was facilitated by forskolin. The lack of agonist effects emphasizes the importance of β -adrenoceptor selectivity in the evaluation of undesirable drug effects. The suggested effects of celiprolol on the bronchial tone observed in patients might be due to a low affinity for the β_2 -adrenoceptor resulting in weak or no antagonism of bronchodilatation evoked by endogenous catecholamines rather than to relevant partial agonist activities. Evaluation of *in vitro* responses at therapeutically active drug-concentrations revealed reasonable therapeutic indices for celiprolol and bisoprolol. Thus, no obvious advantage for celiprolol could be substantiated. The combined interpretation of data from biochemical, functional and pharmacological investigations might provide valuable tools in the investigation of novel drugs for the treatment of pulmonary and cardiovascular disorders which are limited by their adverse effects exerted upon other systems.

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