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ORIGINAL ARTICLE

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The effect of the enantiomers of formoterol on inherent and induced tone in guinea-pig trachea and human bronchus

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Abstract The long-acting β_2-adrenoceptor agonist formoterol is, like all other members of this class of drugs, used as a racemate in the clinic. While the effects of the individual enantiomers have been studied on airway smooth muscle from guinea pig, comparable data on human bronchial smooth muscle are scanty or absent. Therefore, we compared the effects of the enantiomers of formoterol on inherent and induced tone in isolated human bronchi with that on guinea-pig trachea in vitro. The human bronchi either were studied under resting tension conditions or were precontracted with 10 μM carbachol or 0.1 mM histamine. The guinea-pig trachea was precontracted with 0.01, 0.1 or 1 μM carbachol. The racemate and (*R,R*)-formoterol caused a concentration-dependent relaxation of all preparations with an EC_{50} of about 1 nM. In the guinea-pig trachea, the concentration-effect curve for formoterol was moved to the right in response to an increased concentration of carbachol. In both human bronchus and guinea-pig trachea, (*S,S*)-formoterol was more than 1,000 times less potent than (*R,R*)-formoterol. Thus the relaxing effect of formoterol in human airways as well as in guinea-pig trachea was shown to lie with the (*R,R*)-enantiomer. Notably, (*S,S*)-formoterol did not exert any contractile effects within the tested concentration range in either airway preparation. Therefore, we conclude that with regard to relaxant effects the pure (*R,R*)-enantiomer of formoterol does not offer a benefit over the racemate.

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Introduction

Formoterol is a long-acting β_2-adrenoceptor agonist used as a bronchodilator in asthma (Bartow and Brogden 1998). Like all other members of this class of drugs in current clinical use, it comprises a racemic mixture of two enantiomers. Using highly purified enantiomers of formoterol it was shown, on precontracted guinea-pig tracheal strip preparations, that the relaxing effect was confined to the (*R,R*)-enantiomer, whereas the (*S,S*)-enantiomer was virtually inactive (Trofast et al. 1991), the eudismic ratio, i.e., the potency relation between the more active (the eutomer) and the less active (the distomer) enantiomer, being almost 1,000-fold. Similar observations have been made for the (*R*)- and (*S*)-enantiomers of terbutaline (Jeppsson et al. 1984). Experiments with pure enantiomers of formoterol and terbutaline on guinea-pig trachea and main bronchi showed that also the inhibitory effect on cholinergic and non-cholinergic excitatory responses to electrical nerve stimulation was confined to the (*R,R*)- and (*R*)-enantiomers, respectively, with the same high eudismic ratio (Källström et al. 1996).

While the effects of enantiomers of β_2-adrenoceptor agonists have been extensively studied on airway smooth muscle from guinea pig, comparable data on human bronchial smooth muscle are scanty or absent. Therefore, we compared the relaxing capacity of enantiomers of formoterol on human bronchial smooth muscle with that on guinea-pig trachea. In order to explore both potency and efficacy, the effect of formoterol and its enantiomers on induced as well as inherent tone was assessed. Highly purified enantiomers were used to avoid misinterpretation of data (Waldeck 1993).

Materials and methods

The experiments comply with the current laws of the country in which they were performed.

Human bronchus

Tissue preparation. Macroscopically normal bronchial tissues were obtained from nine patients undergoing surgery for lung cancer. Immediately after resection, peripheral airways (2–3 mm internal diameter) were dissected free of alveolar tissue and cut into rings of about 3 mm length.

Tension measurements. The bronchial rings were transferred to 10-ml organ baths containing oxygenated (95% O_2, 5% CO_2) modified Krebs buffer (pH 7.4; 37°C; composition in mM: NaCl 118.4, KCl 4.7, $MgSO_4$ 0.6, $CaCl_2$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.1). To investigate the effect of *rac*-, (*R,R*)- and (*S,S*)-formoterol on resting tension, tissues were equilibrated for about 60 min until a stable resting tension of about 6 mN was achieved. Thereafter, a single dose of the β-adrenoceptor agonist isoprenaline (1 μM) was applied to determine the amount of inherent tone. After washing and reequilibration of the tissues, formoterol concentration-effect curves within a range of 10 pM–30 μM were constructed.

To investigate the effect of *rac*-, (*R,R*)- and (*S,S*)-formoterol on carbachol and histamine-induced tone, tissues were equilibrated for about 60 min at a resting tension of about 4 mN. Thereafter, a single dose of the β-adrenoceptor agonist isoprenaline (1 μM) was applied. After several washings and reequilibration of the tissues, bronchial rings were precontracted with 10 μM carbachol or 0.1 mM histamine. Previous experiments have shown that these concentrations induce approximately 90% of the maximal response (EC_{90}) to the respective stimulus. After 10–20 min the tone stabilized and formoterol concentration-effect curves within a range of 10 pM–30 μM were constructed. Concentration-effect curves were obtained in a cumulative manner, using incremental concentrations spaced at half-$\log_{10}$ intervals.

Analysis of results. All responses were recorded as changes in isometric tension using an eight-channel thermal chart recording system (Lectromed MultiTrace 8; Lectromed, Hertfordshire, UK). The traces were evaluated manually. Relaxing responses to formoterol on resting tension were expressed in percent of the maximal response to isoprenaline (% iso. max.). Relaxing responses of formoterol on carbachol- or histamine-induced tension were expressed in percent of the maximal response to either stimulus (% carb. max., % hist. max.). The potency of *rac*-, (*R,R*)- and (*S,S*)-formoterol was calculated from concentration-effect curves by non-linear curve fitting using the InPlot program (GraphPad Software, San Diego, Calif., USA) for each individual tissue and expressed as the pEC_{50}-value, i.e., $-\log_{10}$ of the concentration of formoterol giving a half-maximal effect. Statistical analysis was performed using paired or unpaired *t*-tests, as appropriate. All values quoted are means ± SEM. *P*-values <0.05 were considered significant for two-tailed comparisons.

Guinea-pig trachea

Tissue preparation. Male Dunkin-Hartley guinea pigs (Charles River, Sweden), 200–400 g, were anaesthetized with pentobarbitone and exsanguinated by cutting the subclavian arteries. The trachea was dissected out, freed from connective tissue and cut into sections comprising two cartilage rings. Silk threads were fastened to the cartilage on each side of the muscle. The cartilage was then cut open ventrally and the strip was mounted in an organ bath. All experiments were performed in water-jacketed organ baths (40 ml) containing oxygenated Krebs solution (37°C; composition in mM: NaCl 118.4, KCl 4.7, $MgSO_4$ 1.16, $CaCl_2$ 2.5, KH_2PO_4 1.18, $NaHCO_3$ 25.0, glucose 11.1; the solution was oxygenated with a mixture of 5% CO_2 in O_2).

Tension measurements. Isometric tension was measured with a Grass force transducer (FTO3). The signals were transformed in an NB-MIO-16L-8 analogue digital converting board and registered in a Macintosh Quadra 700 computer with a data acquisition and evaluation program made with the LabView 2 signal-processing software (National Instruments, Austin, Tex., USA). The preparation, mounted at a basal tone of 5 mN, was allowed to stabilize for 1 h. The viability of the preparation was tested by adding 0.1 μM carbachol to the bath followed 15–20 min later by 3 μM terbutaline. This was followed by a 60-min rinsing and recovery period. Preparations that did not respond with contractile and relaxant effects (less than 10%) were discarded.

At the beginning of the experiments tracheal strip preparations were precontracted with either 0.01, 0.1 or 1 μM carbachol. After 15–20 min the tone stabilized and formoterol concentration-effect curves for either enantiomer were constructed. Concentration-effect curves were obtained in a cumulative manner, using incremental concentrations spaced at $\log_{10}$ intervals. After the final dose of the test compound had been given, maximum relaxation was established by adding 10 μM isoprenaline followed by 1 mM theophylline.

Analysis of results. Relaxant effects were calculated as a percentage of the maximum relaxation induced by theophylline added at the end of the experiment. Concentration-effect curves were constructed and pEC_{50}-values identified for each individual preparation with the aid of the LabView software. Means ± SEM were calculated and statistical evaluation was made with the aid of Student's *t*-test.

Drugs used

Enantiomeric pure (*R,R*)-formoterol fumarate (99.4% pure, containing 0.2% of the (*S,S*)-enantiomer) and (*S,S*)-formoterol fumarate (99.6% pure, containing 0.1% of the (*R,R*)-enantiomer) were synthesized in the laboratories of AstraZeneca (Lund, Sweden). Racemic formoterol fumarate and theophylline were provided by AstraZeneca. (*R*)-Isoprenaline hydrochloride and carbamylcholine chloride (carbachol) were obtained from Sigma Chemical (St. Louis, Mo., USA). Pentobarbitone sodium was obtained from Apoteksbolaget, Sweden.

Results

Effect of formoterol enantiomers on human bronchus

Inherent tone

The resting tension (mean ± SEM) of human bronchial ring preparation was 5.61±0.21 mN (*n*=4). The inherent tone, i.e., the magnitude of relaxation after a single dose (1 μM) of isoprenaline, was 4.31±0.25 mN (*n*=4). After washing and reequilibration the tension stabilized at 5.87±0.35 mN (*n*=4), which was not significantly different from the resting tension before the application of isoprenaline.

Rac-, (*R,R*)- and (*S,S*)-formoterol relaxed inherent tone concentration-dependently while almost no changes could be observed in the tension-control tissues (range: –2% to 5% iso. max.) throughout the entire experiment (Fig. 1). There was no apparent difference in potency or efficacy between the racemate and the pure (*R,R*)-enantiomer in

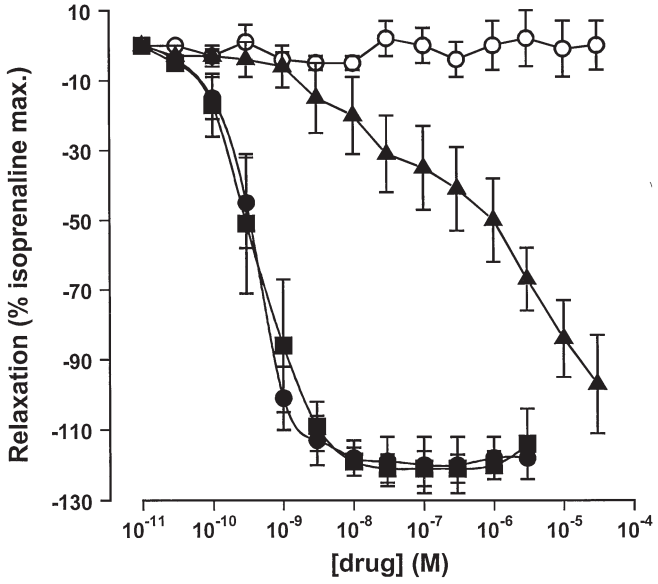

Fig. 1 Stereoselective relaxation of resting tension by formoterol in human bronchial rings. The racemate (●), (*R,R*)- (■) or (*S,S*)-formoterol (▲) were added cummulatively to the organ bath. Control preparations (○) were run in parallel. The data are means ± SEM of four experiments

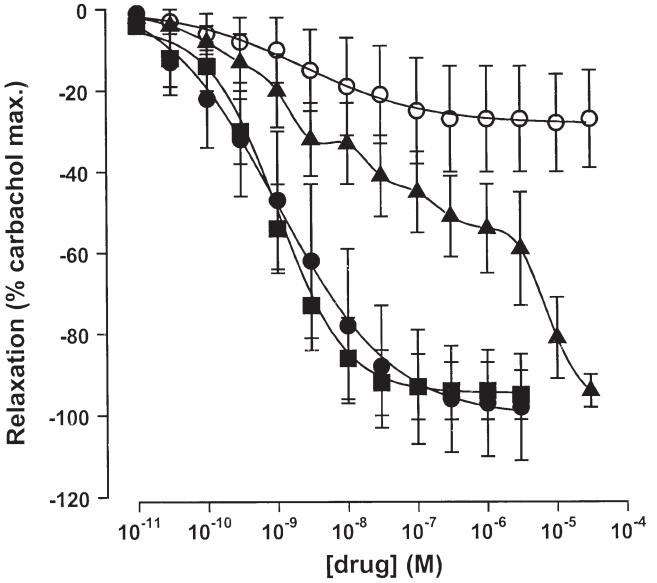

Fig. 2 Stereoselective relaxation by formoterol of carbachol-precontracted human bronchial rings. Bronchial ring preparations were precontracted with 10 μM carbachol. The racemate (●), (*R,R*)- (■) or (*S,S*)-formoterol (▲) were added cummulatively to the organ bath. Control preparations (○) were run in parallel. The data are means ± SEM of four experiments

reducing inherent tone. Whereas (*R,R*)-formoterol was active in the nanomolar range, (*S,S*)-formoterol was at least 1,000 times less potent ($P<0.001$; $n=4$) and did not reach a plateau within the tested concentration range.

Carbachol-induced tone

Carbachol (10 μM, approx. EC_{90}) increased human bronchial tone by about 5.88±0.52 mN to a total tension of 9.49±0.50 mN. *Rac*-, (*R,R*)- and (*S,S*)-formoterol relaxed the precontracted bronchi concentration-dependently. A moderate decrease in the induced tone over time was observed in the control tissues (–28% carb. max.; Fig. 2). The (*R,R*)-enantiomer of formoterol and the racemate almost completely relaxed carbachol-contracted bronchial preparations with a potency similar to that with inherent tone (Fig. 2; Table 1). The (*S,S*)-enantiomer of formoterol was again about 1,000 times less potent than the (*R,R*)-enantiomer and did not reach a plateau within the tested concentration range. The concentration-effect curve of (*S,S*)-formoterol had an atypical appearance with a sharp drop in tone from 3 μM. However, some loss of tension over time – which has been observed in the control tissues – may have contributed to the distortion of this curve. Since the concentration-effect curves of the two enantiomers were not parallel, a valid eudismic ratio could not be properly calculated.

Histamine-induced tone

Histamine (0.1 mM, approx. EC_{90}) increased human bronchial tone by about 6.04±0.77 mN to a total tension of 8.71±0.78 mN. *Rac*-, (*R,R*)- and (*S,S*)-formoterol relaxed the precontracted bronchi concentration-dependently. Only slight changes could be observed in the tension-control tissues (range: –2% to 10% hist. max.; Fig. 3). The (*R,R*)-enantiomer of formoterol and the racemate almost completely relaxed histamine-contracted bronchial preparations (Fig. 3). The concentration-effect curve of (*S,S*)-formoterol was positioned four log units further to the right and did not reach a plateau within the tested concentration range. Thus (*S,S*)-formoterol was 10,000 or more times less potent than (*R,R*)-formoterol in relaxing histamine-induced tone ($P<0.001$; $n=6$)

Neither for the racemate nor for the (*R,R*)-enantiomer were apparent differences found in pEC_{50} between the inherent, carbachol-induced and histamine-induced tone (Table 1). For the (*S,S*)-enantiomer no apparent differences were found in the pEC_{50} between the inherent and carbachol-induced tone; however, the estimated pEC_{50} for the histamine-induced tone appeared to be approximately one $\log_{10}$ unit higher. Furthermore, there was no indication of a bronchospasmogenic effect of (*S,S*)-formoterol on inherent and induced bronchial tone within the tested concentration range.

Effects of formoterol enantiomers on guinea-pig trachea

Carbachol-induced tone

(*R,R*)-formoterol caused a concentration-dependent and complete relaxation of carbachol-precontracted guinea-pig tracheal preparations (Fig. 4). An increase in the carbachol

Table 1 Stereoselective relaxation by formoterol of airway smooth muscle from guinea-pig trachea and human bronchus. The number of tissue preparations for the individual experiments is given *in parentheses*. Values *within brackets* indicate estimated values assuming a maximum relaxation equal to that obtained with (*R,R*)-formoterol

[a]From Källström et al. 1994

Origin of tissue and contractile state	*rac*-Formoterol (pEC_{50})	(*R,R*)-Formoterol (pEC_{50})	(*S,S*)-Formoterol (pEC_{50})	Eudismic ratio
Human bronchus				
Inherent tone	9.48±0.11 (5)	9.49±0.13 (5)	{~6.1 (5)}	{~2,500}
Carbachol 10 μM	8.81±0.24 (7)	9.12±0.12 (7)	{~6.3 (7)}	{~900}
Histamine 0.1 mM	8.89±0.08 (7)	9.32±0.09 (6)	{~5.1 (8)}	{~15,000}
Guinea-pig trachea				
Carbachol 0.01 μM	–	9.80±0.11 (5)	6.45±0.07 (5)	2,240
Carbachol 0.1 μM	–	9.49±0.06 (5)	6.05±0.11 (5)	2,750
Carbachol 1 μM	8.76±0.09 (6)[a]	8.62±0.05 (5)	5.17±0.09 (5)	2,820

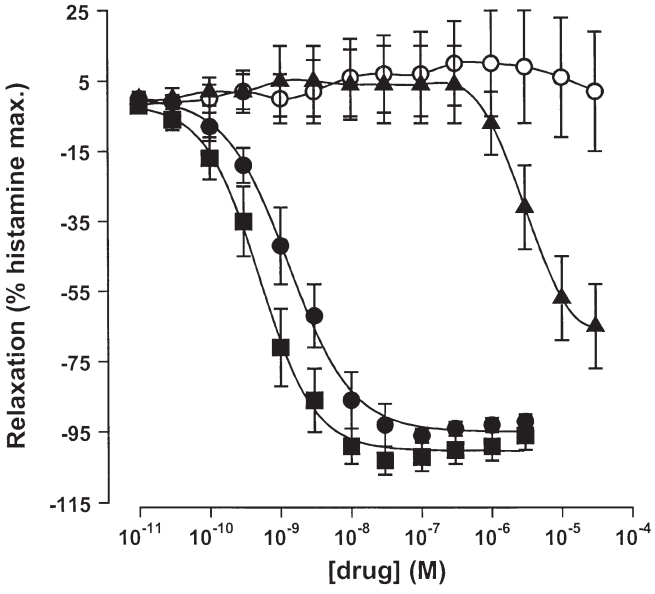

Fig. 3 Stereoselective relaxation by formoterol of histamine-precontracted human bronchial rings. Bronchial ring preparations were precontracted with 0.1 mM histamine. The racemate (●), (*R,R*)- (■) or (*S,S*)-formoterol (▲) were added cummulatively to the organ bath. Control preparations (○) were run in parallel. The data are means ± SEM of six experiments

concentration used for precontraction from 0.01 μM to 1 μM caused a statistically significant right-shift of the concentration-effect curve by more than one log_{10} unit ($P<0.001$); the curve obtained with 0.1 μM carbachol was positioned in between. The pEC_{50}-value for (*R,R*)-formoterol obtained after precontraction with 1 μM carbachol was comparable to the pEC_{50}-value for the *rac*-formoterol during identical experimental conditions in a previous study (Table 1).

There was no difference in the maximal relaxing effects between the (*R,R*)-and the (*S,S*)-formoterol at any occasion. However, the concentration-effect curve of (*S,S*)-formoterol was significantly shifted to the right; the (*S,S*)-enantiomer being 2,000–3,000 times less potent than the (*R,R*)-enantiomer ($P<0.001$). Furthermore, there was no indication of an amplification by (*S,S*)-formoterol of the carbachol-induced tracheal tone. In no case did the maximum relaxation by theophylline exceed that produced by the preceding dose of isoprenaline. Due to the high stability of the carbachol-induced tone in the guinea-pig trachea (Jeppsson et al. 1992), time-matched control preparations were not included in the study.

Discussion

The present study demonstrates that in human isolated bronchus, as well as in guinea-pig trachea, the relaxing effect of the formoterol racemate lies with the (*R,R*)-enantiomer, with a potency ratio of approximately 1,000 over the (*S,S*)-enantiomer. This confirms previous observations on guinea-pig trachea (Trofast et al. 1991) and provides novel information on the human bronchus. It is noteworthy

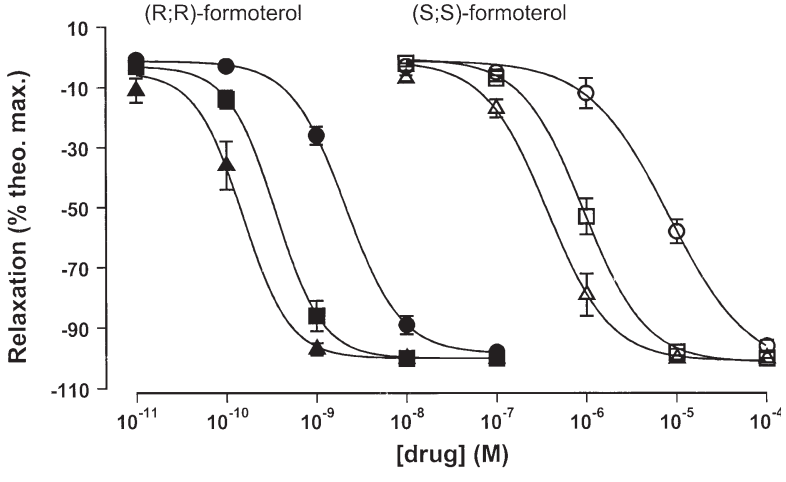

Fig. 4 Stereoselective relaxation by formoterol of carbachol-precontracted guinea-pig tracheal strip preparations. Tone was induced by carbachol, 10 nM (▲,△), 0.1 μM (■,❑) and 1 μM (●,○). (*R,R*)-formoterol or (*S,S*)-formoterol were added cummulatively to the organ bath. Relaxation is expressed as a percentage of the maximum relaxation produced by 1 mM theophylline. Shown are the means ± SEM of five tracheal preparations

thy that (*S,S*)-formoterol did not exert any bronchospasmogenic effects and did not appear to interfere with the relaxing effect produced by (*R,R*)-formoterol in the racemate form.

The racemate of formoterol is a 50/50% mixture of the two enantiomers. Therefore, it would be expected that the (*R,R*)-enantiomer should be twice as potent as the racemate. However, our results suggest that the racemate and the (*R,R*)-formoterol were equipotent, which is most likely explained by the fact that a twofold potency difference lies within the experimental error.

In guinea-pig trachea, the concentration-effect curves for both formoterol enantiomers were shifted to the right in response to an increase in the carbachol-induced tone. The maximum relaxation was unchanged. This pattern would be expected from a system with an excess of available receptors (Kenakin 1987). In contrast, in human bronchial ring preparations, there were no apparent differences between the potencies of either the racemate or the (*R,R*)-enantiomer in relaxing inherent and carbachol- or histamine-induced tone. The experimental conditions did not allow a valid comparison of the maximum relaxation achieved with and without carbachol or histamine. However, a previous study showed that precontraction with 1 μM carbachol did not significantly change the EC_{50} of *rac*-formoterol in human bronchus, but reduced maximal relaxing responses by 15% in comparison to responses in non-precontracted tissues (Molimard et al. 1998). These findings might suggest that under the prevailing experimental conditions in human airways only a few spare receptors are present or that the receptor-effect coupling is low.

A recent observation has demonstrated that formoterol has affinity for muscarinic receptors, antagonistic in nature, with a pK_B of about 5 and with low stereoselectivity (Teschemacher et al. 1998). If this property of formoterol is of functional significance, (*S,S*)-formoterol should be more efficient in relaxing carbachol-contracted rather than histamine-contracted bronchus. Our data point to such a possibility, but the limited material and the poor stability of the carbachol-contracted human bronchus do not permit a closer examination. Previous experiments with (*S,S*)-formoterol on electrically stimulated guinea-pig trachea do not indicate an interaction with the cholinergic system (Källström et al. 1996). However, an anticholinergic component of formoterol in the micromolar range, approx. 10,000-fold higher than those used in the clinic, is not expected to contribute to the therapeutic effect of *rac*-formoterol in nanomolar concentrations.

It has been suggested that the distomers of racemic β_2-adrenoceptor agonists are responsible for induction of the airway hyperreactivity occasionally observed after treatment with this class of drugs (Chapman et al. 1992; Mazzoni et al. 1994). In support of this hypothesis it was shown, on isolated human bronchus, that preincubation with (*S*)-salbutamol increased the contractile response to histamine and leukotriene C_4 (Templeton et al. 1998). The response to allergen was attenuated, however, and the response to a number of other contractile stimuli was unchanged. The clinical studies with the enantiomers of salbutamol available so far do not show a clinically significant airway hyperreactivity after exposure to the (*S*)-enantiomer (Waldeck 1999).

In conclusion, the results of this in vitro study together with previous observations (Trofast et al. 1991; Källström et al. 1996) indicate that pure (*R,R*)-formoterol offers no benefit over the racemate if bronchodilation is the endpoint measured. This conclusion applies both to inherent and induced airway tone. Moreover, our results show that the guinea-pig trachea is a useful surrogate for human bronchial tissue in assessing airway reactivity to enantiomers of β_2-adrenoceptor agonists.

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