Picumeterol: dissociation of improvement in lung function and reduction of airways hyperresponsiveness in asthmatics

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Aims The new potent and selective β_2 -adrenoceptor agonist, GR 114297A (picumeterol) is the R-enantiomer of the racemic form, GR 63411B. Picumeterol has been shown to produce long-lasting relaxation of airways smooth muscle both *in vitro* and *in vivo*. We assessed the intrinsic activity of picumeterol by increasing intracellular levels of c-AMP and compared this with isoprenaline and salbutamol.

Methods In human atopic asthmatics, we have investigated the duration of action and efficacy of picumeterol and GR 63411B with regard to improvement in resting lung function (i.e. FEV₁) and airways responsiveness (i.e. PC₂₀) to methacholine (MCh). The study design consists of two clinical parts each for one drug. Different asthmatics participated in the two studies, seven in the first part and eight in the second part. In human bronchial smooth muscle cells *in vitro*, we have investigated the intrinsic activity of picumeterol in increasing intracellular levels of cyclic AMP and compared it with isoprenaline and salbutamol.

Results *In vivo*, both drugs caused bronchodilatation with similar potency, but, their effects were short-lasting. Despite their bronchodilator activity, neither drug improved PC₂₀, when compared with placebo. *In vitro*, picumeterol was found have intrinsic activity lower than the other β_2 -adrenoceptor agonists tested.

Conclusions In the clinical studies, the bronchodilator potencies of picumeterol and GR 63411B were similar. However, both drugs were short-acting, which is at odds with their activity *in vitro*. Our data suggest that these compounds display dissociation between bronchodilator activity and protection against MCh-induced bronchoconstriction. These findings may be explained by low intrinsic activity and need further conformation.

Keywords: β_2 -adrenoceptor agonist, airway hyperresponsiveness, enantiomer, picumeterol

Introduction

In 1987, salmeterol was first described as a novel, highly potent, and long-acting β_2 -adrenoceptor agonist with a remarkably long duration of action both *in vitro* and *in vivo* [1]. This compound was subsequently shown to cause sustained bronchodilatation and inhibition of methacholine (MCh)-induced bronchoconstriction in asthmatics [2, 3]. We described another highly potent and selective β_2 -adrenoceptor agonist, the racemic GR 63411B and its R-enantiomer, picumeterol [4], which is also longer-acting than salbutamol. Initial preclinical studies suggested that its duration of action may be shorter than that of salmeterol. Nevertheless, picumeterol was 3.8-fold more potent than salmeterol in relaxing guinea-pig trachea, although the intrinsic activity of picumeterol relative to salmeterol was 0.42 [5].

The aim of the present study was to investigate the bronchodilator potency and duration of action of both picumeterol and its racemic form, GR 63411B, in atopic asthmatic subjects. In addition, we also studied their

protective effects against MCh-induced bronchoconstriction. As the intrinsic activity of β_2 -adrenoceptor-agonists is of great importance for both the bronchodilator and protective activity against spasmogens, we assessed the intrinsic activity of picumeterol by increasing intracellular levels of c-AMP and compared this with isoprenaline and salbutamol.

Methods

Subject characteristics

All subjects in the study were atopic asthmatics; there were seven in the first part, and eight different participants in the second part. All subjects had given informed consent. The study was approved by the hospital ethics committee. Patient characteristics are summarised in Table 1. All were non-smokers and had episodic wheezing, consistent with the clinical diagnosis of asthma. They were all clinically stable, without any respiratory infection or exacerbation for 2 months prior to the study. Inhaled corticosteroids were used in a fixed dose up to a maximum dose of 1200 $\mu\text{g day}^{-1}$ for at least 1 month, the dose being constant throughout the study. During the study, salbutamol was used on demand and infrequently with a maximum of 500 μg daily, and not

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12 h immediately before each study day. In study part one: the mean salbutamol dose was 200 µg on demand with a range from 500–0 µg. In study part two: the mean salbutamol dose was 300 µg on demand with a range from 500–0 µg. All subjects showed a reversibility in FEV₁ of at least 15% from baseline value following inhalation of 400 µg salbutamol via Diskhaler®. Finally, they were selected on the presence of airway hyperresponsiveness to MCh (PC₂₀ < 2.5 mg ml⁻¹). FEV₁ values at the start of every study day had to be within 10% of each other, and were ≥ 65% of the predicted value.

Study design

The study consisted of two clinical parts and an *in vitro* part. Both clinical parts of the study were performed in a randomized, double-blind, placebo-controlled, cross-over manner. In both clinical study parts different asthmatic subjects participated, thus a randomized, parallel study design was created for the time point 2 h after inhalation. FEV₁ and PC₂₀ MCh had to be stable throughout the study, i.e. within 10% or 0.5 doubling dose, respectively.

In the first part of the study, each subject received 25, 50 or 100 µg GR 63411B or placebo on 4 separate study days. At the start of each study day (08.00 h), FEV₁ and a methacholine challenge test (PC₂₀ MCh) were performed. The study drug was administered at 12.00 h as a dry powder via a Diskhaler®. FEV₁ and PC₂₀ were measured at 4, 16 and 24 h after inhaling GR 63411B.

In the second part of the study, each subject received 25 or 50 µg picumeterol or placebo on 3 separate study days. At the start of each study day (at 08.00 h), FEV₁ and PC₂₀ MCh were measured. The study drug was then administered as in the first part of the study. FEV₁ values and PC₂₀ MCh were performed 2 and 9 h after inhaling picumeterol.

In the *in vitro* study, we have investigated the intrinsic activity of picumeterol and compared it with isoprenaline and salbutamol in increasing intracellular levels of cyclic AMP in cultured human bronchial smooth muscle cells.

Materials and Methods

Airway responsiveness was assessed by means of a MCh bromide provocation test (PC₂₀ MCh) as was described previously [6]. Briefly, after measuring a baseline FEV₁ with a dry spirometer (Vicatest5 Mijnhardt), a PC₂₀ MCh was measured using a 2 min tidal breathing method adapted from that of Cockcroft *et al.* [6]. Doubling concentrations of MCh bromide from 0.038 mg ml⁻¹ to 9.6 mg ml⁻¹ were inhaled at 5 min intervals. MCh bromide was stored at 4° C and nebulized at room temperature. Solution output was 0.13 ml min⁻¹, using a De Vilbiss 464 nebuliser (DeVilbiss, Somerset, Pennsylvania 15501) connected to a valve box. One value of FEV₁ was measured 30 and 90 s after each inhalation. The inhalations were stopped as soon as the fall in FEV₁ was 20% or more of the baseline FEV₁, or when the upper dose of 9.6 mg ml⁻¹ had been reached. The PC₂₀ was calculated by linear interpolation of the last two points of the log dose-response curve.

Human bronchial smooth muscle cells isolated from primary bronchus were obtained from Dr S. Hirst, St Thomas' Hospital, London. Isolation and initial culture of the cells was carried out by Dr S. Hirst. We received the cells at passage 2 and they were subsequently subcultured at Glaxo Wellcome. Bronchial cells were obtained by the method described by Twort & van Breeman [7, 8]. Briefly, human bronchial smooth muscle was obtained during lung resections from a 56 year old male patient undergoing surgery for carcinoma of the bronchus. Following removal of the epithelium, portions of smooth muscle not invaded by the carcinoma were dissected free of adherent connective and parenchymal tissue. Cells were obtained by enzymatic digestion and cultured [9].

Cells were grown on 48-well tissue culture plates in Dulbecco's modified Eagle's medium containing 10% (v/v) foetal calf serum (FCS), 2 mM L-glutamine, non-essential amino acids (NEAA) and antibiotics maintained at 37° C in 5% CO₂ in air. Cells were cultured in FCS- and NEAA-free medium 24 h prior to the cAMP assay. Cultures were

	GR 63411B	Picumeterol
Number	7	8
Age (years)	32 (6.9)	33 (7.8)
Inhaled corticosteroids (µg)	600 (1200–300)	600 (1200–300)
β ₂ -adrenoceptor agonists (µg) on demand	200 (500–0)	300 (500–0)
Baseline FEV ₁ (% predicted)		
Placebo	78.2 (14.3)	84.5 (14.3)
25 µg	77.6 (16.6)	85.3 (12.4)
50 µg	74.5 (12.1)	83.1 (13.9)
100 µg	78.8 (17.1)	
PC ₂₀ MCh*		
Placebo	0.26 (0.06–0.95)	0.74 (0.11–2.40)
25 µg	0.31 (0.10–1.77)	0.65 (0.06–1.82)
50 µg	0.28 (0.08–1.15)	0.82 (0.11–4.72)
100 µg	0.35 (0.09–1.21)	
Reversibility [#]	18.9 (3.2)	17 (2.1)

Values are means (s.d.). *geometric mean (range).

[#]Reversibility as change in FEV₁ and expressed in % baseline after 400 µg salbutamol (Diskhaler® inhaler).

Table 1 Subjects characteristics

incubated with 125 ml of 10 mM Hepes buffer, pH 7.2, containing 125 mM NaCl, 5 mM KCl, 1.8 mM CaCl_2 , 2 mM MgCl_2 , 0.5 mM NaHCO_3 , 10 mM glucose and 1 mg ml⁻¹ bovine serum albumin before addition of 25 ml of vehicle of each agonist for 10 min at 37°C. At the end of this period, the incubation medium was removed and the assay terminated by the addition of 250 ml of cold 0.2 M HCl. Cells were stored at -70°C. Cyclic AMP concentrations were measured after neutralisation of thawed samples by radioimmunoassay kit (Dupont NEN). Results were expressed as pmol of cAMP per 10⁵ cells. Each agonist was tested at a range of concentrations, and each concentration was tested in three separate wells in a single experiment.

Data analysis

All FEV₁ values were expressed as percentage change from baseline FEV₁ value, which was measured at the start of each study day. The distribution of PC₂₀ values was normalized by logarithmic transformation before statistical analysis. FEV₁-values were then transformed to one single area under the curve (AUC) value, as calculated by trapezoidal method using identical intervals between measurements [10]. Maximum effects of treatment on changes in FEV₁ and PC₂₀ were tested using Student's paired *t*-test after checking for normal distribution. Data are presented as mean values (s.e. mean), a two-sided *P*-value of *P* < 0.05 being significant.

Results

No significant differences in baseline FEV₁ and PC₂₀ MCh were found at the beginning of every visit for the two studies (Table 1). One patient in the second study was withdrawn on placebo day because of an asthma exacerbation.

GR 63411B

The AUC-FEV₁ increased for all three doses, but was only significantly different from placebo for 25 µg. FEV₁ (expressed as percentage baseline) increased significantly 4 h after inhaling the study drug when compared with placebo (Figure 1). The mean treatment difference *vs* placebo at 4 h time point was 10.3(3)% (2.2–18.4, 95% confidence interval) for 25 µg, 13.5(6)% (-0.2–27.1) for 50 µg, and 11.5(9)% (-9.7–32.7) for 100 µg. Mean (s.e. mean) increase in FEV₁

values at 4 h after administration was 18(5)% by 25 µg, 21(3)% by 50 µg, and 19(9)% by 100 µg. The PC₂₀ MCh was not affected by GR 63411B at that timepoint (Figure 1). The individual data are shown in Figure 3a for these drug-induced changes in FEV₁ *vs* PC₂₀ MCh. FEV₁ values expressed as percentage predicted (Table 2) were still below 100% predicted, although a maximum response of each drug was reached. No significant differences between the doses were observed for either FEV₁ values or PC₂₀ MCh values. The circadian pattern of airways calibre and airways hyperresponsiveness was still detectable, though on a higher level compared with placebo.

Picumeterol

The AUC-FEV₁ increased for both doses, but was only significantly different from placebo for 50 µg. FEV₁ (expressed as percentage baseline) increased significantly 2 h after inhaling the study drug when compared with placebo (Figure 2). The mean treatment difference *vs* placebo at 2 h time point was 11.2(2)%, (5.7–21.0, 95% confidence interval) for 25 µg, and 13.4(3)%, (0.7–17.6) for 50 µg. The drug-induced increases in the FEV₁ values at 2 h after administration were 13.3(3)% by 25 µg picumeterol, and 18(4)% by 50 µg picumeterol. Mean PC₂₀ MCh was not altered by either dose of picumeterol at this time point. The individual data are shown in Figure 3b for these drug-induced changes in FEV₁ *vs* PC₂₀ MCh. FEV₁ values expressed as percentage predicted (Table 2) were still below 100% predicted, although a maximum response of each drug was reached. No significant differences between the doses were observed for either FEV₁ values or PC₂₀ MCh values.

No significant differences in improvement of FEV₁ were observed comparing the equivalent doses of GR 63411B with picumeterol. For GR 63411B, an increase of 20(4)% was seen 2 h after inhaling of 25 µg, and of 21(5)% for 50 µg. For picumeterol, an increase of 13(3)% was seen for 25 µg and an increase of 18(4)% for 50 µg 2 h after inhalation.

Adverse effects

In the first part of the study, one patient taking GR 63411B (100 µg) had tachycardia, which was considered to be drug-related. No other serious drug-related events were reported on treatment with GR 63411B. For picumeterol, some

Table 2 Changes in FEV₁ expressed as percentage predicted (95% confidence limits) by both drugs

	GR 63411B				GR 114297 (picumeterol)		
	Placebo	25 µg	50 µg	100 µg	Placebo	25 µg	50 µg
Baseline	78.2 (64–91)	77.6 (62–92)	74.5 (63–85)	78.8 (62–94)	85 (71–98)	85 (75–95)	82 (71–93)
2 h					88 (76–100)	96** (87–105)	96** (87–106)
4 h	83.6 (69–98)	92.2* (76–104)	89.7 (77–101)	91.2* (78–103)			
9 h					92 (77–105)	94 (83–104)	92 (80–105)
16 h	74.6 (61–87)	79.1 (63–94)	75.4 (59–91)	77.1 (66–87)			
24 h	80.9 (66–95)	84.1 (68–99)	77.5 (57–98)	85.2 (72–97)			

P* < 0.05, *P* < 0.001 *vs* placebo.

Figure 1 Effects of GR 63411B on basal levels of FEV₁ (upper panel) and PC₂₀ MCh (lower panel). Upper panel: FEV₁ values measured 4 h before, and 4, 16 and 24 h after inhalation of placebo (■) or GR 63411B at 25 µg (○), 50 µg (●) or 100 µg (▲). Lower panel: log PC₂₀ MCh determined 4 h before, and 4, 16 and 24 h after inhalation of placebo (solid columns) or GR 63411B at 25 µg (open columns), 50 µg (obliquely hatched columns) or 100 µg (vertically hatched columns). Despite the significant increase in FEV₁ (mean, s.e. mean) 4 h after inhaling GR 63411B, no improvement in PC₂₀ was seen, when compared with effects on the placebo-treated group

Figure 2 Effects of picumeterol on basal levels of FEV₁ (upper panel) and PC₂₀ MCh (lower panel). Upper panel: FEV₁ values measured 2 h before, and 2 and 9 h after inhalation of placebo (■) or picumeterol at 25 µg (○) or 50 µg (●). Lower panel: log PC₂₀ MCh determined before and after inhalation of placebo (solid columns) or GR 63411B at 25 µg (open columns) or 50 µg (vertically hatched columns). Despite the significant increase in FEV₁ (mean, s.e. mean) 2 h after inhaling picumeterol, no improvement in PC₂₀ was seen, when compared with effects on the placebo-treated group. * $P < 0.05$; ** $P < 0.01$

events were reported, such as tachycardia, hyperactivity, tremor, agitation, which were also considered to be drug-related. In one patient, these mild adverse effects were observed at doses of 25 and 50 µg, and in another patient, only at 50 µg. No other drug-related side-effects were observed.

Human bronchial smooth muscle

Isoprenaline (0.0001–1.0 mM), salbutamol (0.1–10 mM) and picumeterol (0.0001–0.03 mM) all caused concentration-

related increases in intracellular levels of cyclic AMP (Figure 4). Differences in maximum attained responses to the three agonists were observed, although higher concentrations of isoprenaline (10 mM) and picumeterol (0.1–1.0 mM) caused no further increase in cyclic nucleotide levels. Thus, isoprenaline caused the greatest maximal increase, being of the order of 40 pmol cyclic AMP/10⁵ cells. In contrast, picumeterol caused a much lower maximal increase, being 4.9-fold lower than that obtained with isoprenaline. Salbutamol failed to produce a plateau response even at the highest concentration tested (10 mM), and thus

Figure 3a and b Maximum treatment induced changes in PC_{20} MCh (expressed in doubling doses) *vs* FEV_1 (expressed in % baseline). Figure 3a shows the scatterplot 4 h after inhaling of 25 μ g (■), 50 μ g (●), and 100 μ g (▲) GR 63411b. Figure 3b shows the scatterplot 2 h after inhaling of 25 μ g (■) and 50 μ g (●) picumeterol

it was not certain that a true maximum response had been achieved with this agonist in these cells (Table 3). Despite this, responses obtained with the highest concentration of salbutamol were at least twice the maximum achieved with picumeterol.

Discussion

This study was designed to investigate the bronchodilator and anti-bronchoconstrictor effects of picumeterol and its racemate, GR 63411B, in atopic asthmatic subjects. The results show that both compounds produce a clear bronchodilatation, i.e. an increase in FEV_1 for up to 2 and 4 h after administration of picumeterol and GR 63411B respectively.

The experiments with picumeterol on cyclic AMP levels in human bronchial smooth muscle cells *in vitro* revealed that it is highly potent in increasing cyclic AMP. But it is apparently of low intrinsic activity, causing a maximum elevation of cyclic AMP levels of the order of only 20% of that to isoprenaline. As a maximum response to salbutamol was not established, it is not possible to determine with any

Figure 4 Human bronchial smooth muscle cells. Concentration-effect curves to isoprenaline (▲), salbutamol (●) and picumeterol (△) for increases in intracellular levels of cyclic AMP. Each point is the mean of three determinations from a single experiment, vertical bars represent s.d.

Table 3 Human bronchial smooth muscle cells. Potency values of the increase of cyclic AMP of isoprenaline, salbutamol and picumeterol. Potency values are expressed as geometric means (95% confidence limits). The intrinsic activity is calculated from the maximal point of plateau, arithmetic mean (95% confidence limits). Data are from at least three experiments

β -adrenoceptor agonist	Maximum response#	s.d.	Intrinsic activity compared with isoprenaline	n
Isoprenaline	42.2	3.3	100%	3
Salbutamol	22.7*	3.1	52.9% (42.4–63.4)	3
Picumeterol	8.6	0.8	21.3% (18.9–23.7)	3

#(pmol cAMP/ 10^5 cells), * E_{max} is only an estimate at the highest concentration.

confidence the maximal response to picumeterol relative to salbutamol, nevertheless, the response achieved at the highest concentration of salbutamol tested was nearly three-fold greater than the maximum response with picumeterol. It is known that salbutamol has a lower efficacy than isoprenaline, and under the appropriate conditions, it can behave as a partial agonist [11]. The present study suggests that the maximal efficacy of picumeterol is substantially lower even than that of the highest used concentration of salbutamol.

Despite the clear increase in FEV_1 with both GR 63411B and picumeterol, no significant reduction in airways responsiveness to MCh was obtained. This remarkable finding is in contrast with the well-reported protective effects of both short- and long-acting β_2 -adrenoceptor agonists on airways hyperresponsiveness; a single bronchodilator dose of inhaled β_2 -adrenoceptor agonists has been reported to cause a shift

in the dose-response curve to MCh of between two and four doubling doses [12, 13]. In our study, we did not have a positive control i.e. inhaled salbutamol to show effects on FEV₁ and PC₂₀ MCh in this group of asthmatics. The lack of effect of picumeterol on airways responsiveness suggests that it may not solely be a function of bronchodilatation that β_2 -adrenoceptor agonists protect against airways responsiveness but of functional antagonism as well. Alternatively, both parts of the study contained a small number of participants and therefore a more variable effect on FEV₁ and PC₂₀ MCh has been observed which may explain partly the observed dissociation between bronchodilation and airways hyperresponsiveness. All of the participants of the study used β_2 -adrenoceptor agonists on demand for symptom relief, although they were in a stable phase of their disease. Regular treatment of β_2 -adrenoceptor agonists can induce tolerance for bronchoprotection [14, 15]. Since this has never been found for on demand use, we suggest that this phenomenon may not be an explanation for our observation.

This highly potent β_2 -adrenoceptor agonist (40-fold more potent *in vitro* than isoprenaline), picumeterol, exhibits a low intrinsic activity. Therefore, we hypothesize that clinically, such a partial β_2 -adrenoceptor agonist can relax airways smooth muscles, as demonstrated by an increase in FEV₁, but that this relaxation is insufficient to give reduction of MCh-induced bronchoconstriction. It is believed that an increase in intracellular cyclic AMP is necessary for relaxation of airway smooth muscle by β_2 -adrenoceptor agonists, but that the increase possible with an agonist of the efficacy of picumeterol, while sufficient to inhibit basal tone, is insufficient to reverse a MCh-induced contraction. There is evidence that methacholine-induced contractions of airways smooth muscle are more resistant to reversal than are those to other spasmogens such as histamine and leukotrienes, and that in the presence of high concentrations of methacholine, even isoprenaline can behave as a partial agonist [16, 17]. It is important to note that salmeterol has also been reported to be a rather low efficacy β_2 -adrenoceptor agonist [11], but despite this, it produces both bronchodilatation and inhibition of MCh-induced bronchoconstriction in a clinical setting. It may well be that the intrinsic activity of salmeterol is low relative to isoprenaline and even salbutamol, but it is higher than that of picumeterol.

While the R-enantiomer, picumeterol, produced bronchodilatation of similar degree to that of the racemic form, GR 63411B, we did not observe a dose-dependent bronchodilator effect with either agonist. While this may be a characteristic of the agonist activities of these compounds, it is also possible that in both parts of the study, the doses used were too high to demonstrate a submaximal bronchodilatation. Contrary to the preclinical findings [4], no long-lasting bronchodilatation was observed with either drug. Although picumeterol has been shown to be long-acting both *in vitro* and *in vivo* in animal studies, in neither case was the duration as long as that for salmeterol [18, 19]. When given to man, salmeterol is a long-acting bronchodilator with a duration of action of at least 12 h, whereas the effects of GR 63411B and picumeterol last for no longer than 4 h. As the duration of clinical bronchodilator activity of picumeterol is shorter than that previously reported with salmeterol, the present findings may not seem unexpected.

However, the observation that picumeterol was not longer acting than salbutamol is hard to understand, while in preclinical studies it was substantially longer acting than salbutamol, although shorter acting than salmeterol.

In summary, we conclude that the R-enantiomer, picumeterol, is of similar potency to the racemic form, GR 63411B, and both compounds are clinically short-acting, which contrasts with *in vitro* and *in vivo* animal studies. This may suggest species differences in responses to β_2 -adrenoceptor stimulation. Furthermore, we observed a single dose of a β_2 -adrenoceptor agonist demonstrating a bronchodilator effect without concurrent protection against MCh-induced bronchoconstriction. We suggest that this may be due to an efficacy of the compound sufficient to cause bronchodilatation, but insufficient for protection against MCh-induced bronchoconstriction. Since no positive control was incorporated further studies are necessary to confirm this observation.

References

- 1 Bradshaw J, Brittain RT, Coleman RA, *et al.* The design of salmeterol, a long-acting selective β_2 -adrenoceptor agonist. *Br J Pharmacol* 1987; **92**: 590P.
- 2 Ullman A, Svedmyr N. Salmeterol, a new long-acting inhaled β_2 adrenoceptor agonist: comparison with salbutamol in adult asthmatic patients. *Thorax* 1988; **43**: 674–678.
- 3 Derom EY, Pauwels RA, Van der Straeten ME. The effect of inhaled salmeterol on methacholine responsiveness in subjects with asthma up to 12 hours. *J Allergy Clin Immunol* 1992; **89**: 811–815.
- 4 Nials AT, Coleman RA, Johnson M, Vardey CJ. GR 114297A: a potent and long-acting β_2 -adrenoceptor agonist. *Eur Respir J* 1993; **6**: 589s.
- 5 Ullmann MA, Man Y, Coleman RA, Vardey CJ. The effect of picumeterol, a novel long-acting, selective β_2 -adrenoceptor agonist. *Am J Respir Crit Care Med* 1995; **151**: A822.
- 6 Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977; **7**: 235–243.
- 7 Twort CHC, van Breeman C. Human airway smooth muscle in culture. *Tissue Cell* 1988; **20**: 339–344.
- 8 Twort CHC, van Breeman C. Human airway smooth muscle in culture; control of intracellular calcium store. *Pulmonary Pharmacology* 1989; **2**: 45–53.
- 9 Hirst SJ, Barnes PJ, Twort CHC. Quantifying proliferation of cultured human and rabbit airway smooth muscle cells in response to serum and platelet-derived growth factor. *Am J Respir Cell Mol Biol* 1992; **7**: 574–581.
- 10 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurements. *Lancet* 1986; **ii**: 307–310.
- 11 Dougall IG, Harper D, Jackson DM, Leff P. Estimation of the efficacy and affinity of the β_2 -adrenoceptor agonist salmeterol in guinea-pig trachea. *Br J Pharmacol* 1991; **104**: 1057–1061.
- 12 Magnussen H, Rabe KF, Nowak D. Therapy of bronchial hyperresponsiveness. *Clin Exp Allergy* 1991; **21**: 379–389.
- 13 Campos Gongora HJ, Wisniewski A, Tattersfield AE. A single-dose comparison of inhaled albuterol and two formulations of salmeterol on airway reactivity in asthmatic subjects. *Am Rev Respir Dis* 1991; **144**: 626–629.
- 14 Kraan J, Koëter GH, van de Mark TW, Sluiter HJ, de Vries K. Changes in bronchial hyperreactivity induced by 4 weeks of treatment with antiasthmatic drugs in patients with

- allergic asthma: a comparison between budesonide and terbutaline. *J Allergy Clin Immunol* 1985; **76**: 628–636.
- 15 Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dijkman JH, Sterk PJ. Long-term effects of a long-acting beta 2-adrenoceptor agonist, salmeterol, on airway hyperresponsiveness in patients with mild asthma. *N Engl J Med* 1992; **327**: 1198–1203.
- 16 Torphy TJ, Zheng C, Peterson SM, Fiscus RR, Rinard GA, Mayer SE. Inhibitory effect of methacholine on drug-induced relaxation, cyclic AMP accumulation, and cyclic AMP-dependent protein kinase activation in canine tracheal smooth muscle. *J Pharmacol Exp Ther* 1985; **233**: 409–417.
- 17 Fedan JS, Nutt ME, Frazer DG. Reactivity of isolated guinea-pig trachea to methacholine histamine and isoproterenol applied serosally versus mucosally. *Eur J Pharmacol* 1990; **190**: 337–345.
- 18 Ball DI, Brittain RT, Coleman RA, et al. Salmeterol, a novel, long-acting β_2 -adrenoceptor agonist: characterization of pharmacological activity *in vitro* and *in vivo*. *Br J Pharmacol* 1991; **104**: 665–671.
- 19 Nials AT, Sumner MJ, Johnson M, Coleman RA. Investigations into factors determining the duration of action of the β_2 -adrenoceptor agonist, salmeterol. *Br J Clin Pharmacol* 1992; **33**: 507–515.

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