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The effects of formoterol, a long-acting β_2-adrenoceptor agonist, on mucociliary activity

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Abstract

The effect on mucociliary function of formoterol, a β_2-adrenoceptor agonist bronchodilator with a prolonged duration of action (as compared with salbutamol or terbutaline), was investigated both in vitro and in vivo with a photoelectric technique. Formoterol, and its (*R*,*R*)-enantiomer, increased ciliary beat frequency in vitro in guinea pig trachea preparations (peak increase $17.2 \pm 2.0\%$ at a concentration of 10^{-7} M) and in vivo in the rabbit maxillary sinus (peak increase $23.0 \pm 4.0\%$ at a dosage of 1 nmol/kg). Formoterol was approximately 100 times more potent than terbutaline in vitro, as judged from the dose-response curve. The main difference between their effect in vivo was the 2-fold longer duration of the mucociliary acceleration after formoterol at 1 nmol/kg than after terbutaline at the equi-effective dosage of 10 nmol/kg terbutaline (20 vs. 10 min, respectively). The findings indicate formoterol to be a powerful, long-acting ciliostimulant, a property which may be of clinical advantage in the treatment of airway disease.

Keywords: Cilia; Formoterol; Terbutaline; Trachea; Maxillary sinus; Mucociliary clearance

1. Introduction

Terbutaline and salbutamol are common β_2-adrenoceptor agonist bronchodilators which have been in use for the symptomatic treatment of asthma for almost two decades. The main drawback of these two drugs is their short duration of action, which is too brief to control nocturnal asthma or for convenient maintenance treatment. Formoterol, or ((+(*R**;*R**)-(*N*-[2-hydroxy-5-[1-hydroxy-2[[2-(*p*-methoxyphenyl)-2-propyl]amino]ethyl]phenyl]formamide, is known to be a potent β_2-adrenoceptor agonist (Ida, 1976) and to have a longer duration of action than terbutaline when inhaled (Löfdahl and Svedmyr, 1986). Like most other bronchodilators, formoterol is structurally related to isoprenaline. The structural configuration of the formoterol molecule allows four different stereoisomers, and the generic name formoterol refers to the compound's racemic mixture (the enantiomeric mixture *R*,*R* + *S*,*S*).

Besides their bronchodilator action, β-adrenoceptor agonists have other pharmacological properties of clinical importance. The mucociliary system is the major mechanism for maintaining the tracheobronchial airways free from mucus during airway disease, and β_2-adrenoceptor agonists have pronounced effects on the mucociliary system in contrast to β_1-adrenoceptor agonists, which have no effect on mucociliary activity (Hybbinette and Mercke, 1982a). β_2-Adrenoceptor agonists accelerate mucociliary activity both in vitro and in vivo (Hybbinette and Mercke, 1982a; Wong et al., 1988a,b; Yanaura et al., 1981). Their mucociliary effect may be of clinical benefit, since mucociliary transport is impaired in asthma and further decreased after bronchial provocation with allergen (Mezey et al., 1978). This view is supported by the report that mucociliary transport is enhanced by terbutaline both in healthy subjects and in patients with obstructive lung disease (Sackner et al., 1976).

In view of the fact that formoterol is intended for inhalation therapy in asthma, it was considered of

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interest to study its effects on mucociliary activity both in vitro and in vivo, and to compare the effects of formoterol on the mucociliary response to those of its enantiomers and terbutaline.

2. Materials and methods

2.1. In vitro experiments

Guinea pigs weighing 200–300 g were used. Immediately after the animals were killed by a blow to the head, the trachea along with the main bronchi were removed and placed in aerated Krebs solution. Connective tissues were carefully removed and the trachea was cut into small rings of about 0.5–0.8 mm with the help of a dissecting microscope.

Adherent mucus was washed off with Hepes (*N*-2-hydroxyethylpiperazine, *n*-2-ethane, sulphonic acid) buffer. The tracheal preparation was placed in a drop of Hepes buffer and transferred to a coverslip, which was then inverted on a glass slide, 2 mm thick with a 1.2 mm deep cavity in the centre, thus providing a preparation freely suspended in a drop of Hepes buffer. The suspended drop preparation was placed on a microscopic stage, maintained at room temperature (20–21°C), and the beating cilia were viewed at 400 × magnification in a Nikon-Optiphoto CF (chromatic aberration free) microscope (Khan et al., 1986).

The cilia were oriented to interrupt the passage of light through a slit in a diaphragm (0.2 mm) into the photometer (Nikon photometer P1), which transduced the light energy to an electrical signal. The electrical signal thus generated was in turn converted into a reading of ciliary beat frequency displayed on the screen of a Nicolet 3091 oscilloscope. The signals displayed on the oscilloscope were also recorded on paper by means of an X-Y BD recorder. Ciliary beat frequency in each preparation was recorded from six different sites and a mean frequency was calculated, the results being expressed as means ± S.E.M. of 8–15 different preparations.

Solutions

Hepes buffer had the following composition (in mM): NaCl 135, KCl 4.6, $MgSO_4$ 1.2, $CaCl_2$ 1.5, glucose 11, Hepes 10. The pH was adjusted to 7.4 with Tris(hydoxymethyl)aminomethane.

Krebs buffer had the following composition (in mM): NaCl 120, KCl 4.0, $NaHCO_3$ 20, $NaHPO_4$ 1.5, $MgSO_4$, $CaCl_2$, glucose 10, bubbled with 95% O_2 and 5% CO_2.

Formoterol, its enantiomers (*R*,*R*) and (*S*,*S*), terbutaline (Astra Draco, Sweden) and the specific β_2-adrenoceptor antagonist propranolol (Sigma, St. Louis, MO, USA) were dissolved in Hepes buffer and the pH adjusted to 7.4.

Experimental procedure

After equilibration for 30 min, the baseline level of ciliary beat frequency was recorded. The ring preparation was then immersed in different (usually increasing) concentrations of the various compounds studied. Ciliary beat frequency was again recorded after 15 min of exposure.

In the blockage experiments with propranolol, 10^{-5} M of this compound was added to the bath 15 min prior to experiments with formoterol.

2.2. In vivo experiments

The experiments were performed on New Zealand rabbits of either sex, weighing 2.6–3.4 kg. Details of the anaesthetic and surgical techniques used have been published previously (Hybbinette and Mercke, 1982b). The animals were anaesthetized with urethane 2 g/kg i.m. as an initial dose, an extra dose of 0.5 g/kg being given i.v. during the operation. Drugs were administered via a retrograde cannula in the facial or lingual artery, continuously perfused with saline (2–5 ml/h). The substances given were dissolved in physiological saline. The mucosa in the maxillary sinus was exposed through a trepanation of about 2 × 8 mm, which was immediately covered with an anti-mist window sealed to the bone with bone wax (Ethicon, UK).

The mucociliary activity (visible as flickering light reflections) was observed through a binocular microscope. The criterion of a properly functioning preparation was visible transportation of such small particulate matter as mucus clumps and shed cells. One of the eyepieces was then exchanged for a phototransducer and the mucociliary activity was recorded photoelectrically. The mucociliary wave pattern was monitored continuously on an oscilloscope and recorded on an ink writer during challenge. The recordings were analyzed by a computerized frequency calculator, which calculated the mucociliary wave frequency in waves/min every 10 s during challenge and at intervals of 1–5 min otherwise. The frequency changes induced were expressed as percentages of the basal mucociliary wave frequency (frequency zero level) immediately preceding drug administration. ECG and rectal temperature were monitored, and body temperature was maintained at 37.0–38.5°C with a heating pad.

Formoterol and terbutaline (ASTRA-Draco, Sweden) were dissolved in physiological saline at a concentration of 10^{-3} M. This stock solution was kept in a refrigerator at 5°C and further dilutions were made in saline.

Experimental procedure

Before the experiments began, mucociliary activity was recorded once a minute until the mucociliary wave frequency had stabilized (usually a period of 30 min).

The following series of experiments were run: (1) Eight rabbits were challenged with increasing dosages of formoterol (10 pmol/kg, 100 pmol/kg, 1 nmol/kg, 10 nmol/kg and 100 nmol/kg) injected as bolus doses of 0.1 ml/kg given over 3 s and followed by 0.3 ml saline to flush out the cannula. Injections with physiological saline of 0.1 ml/kg served as controls. The time lapse between different doses of formoterol was at least 30 min. (2) In an extra series, six additional rabbits were challenged with 10 and 100 nmol/kg formoterol with a time lapse of 2 h. (3) Seven rabbits were challenged with bolus injections of 0.1 nmol/kg and 1 nmol/kg of formoterol followed by 1 nmol/kg and 10 nmol/kg terbutaline. The time lapse between the various injections was at least 30 min. In three of these rabbits the order of injections was reversed (i.e., terbutaline being given before formoterol).

2.3. Statistics

Dose-response and time-course curves were plotted from the maximum change in mucociliary activity during the first 10 min after challenge. The results are expressed as means and standard error of the mean (S.E.M.) except for the frequency zero levels (baseline mucociliary activity before a challenge), which are given as means and standard deviations (S.D.). Each preparation (in vitro) and each rabbit (in vivo) served as its own control whenever possible. The paired t-test was used for statistical evaluations which were based on values for maximum response and area under the curve (AUC). This parametric test was chosen since the distribution of the response values was found to be reasonably consistent with a Gaussian distribution, with the exception of the values for in vivo duration, which were not normally distributed and Wilcoxon's rank sum test was therefore used for the comparison of durations instead of the t-test. P-values < 0.05 were considered significant.

3. Results

3.1. In vitro experiments

Formoterol caused a moderate but statistically significant increase of the ciliary beat frequency in the guinea pig trachea (Fig. 1). The maximum increase in ciliary beat frequency, $17.2 \pm 2\%$, was seen with 10^{-7} M formoterol. The effect of terbutaline was similiar to that of formoterol, but the concentration-response curve was roughly two log units to the right. The maximum change of ciliary beat frequency produced by terbutaline was an increase of $23.7 \pm 4.1\%$ at the highest concentration tested (10^{-3} M). In the presence of propranolol 10^{-5} M, the effect of 10^{-7} M formoterol was abolished. The ciliary beat frequency was 8.0 ± 0.8 Hz after propranolol and 8.0 ± 0.7 Hz after formoterol was added to the bath ($n = 17$). Propranolol itself had no effect on ciliary beat frequency, the initial ciliary beat frequency being 8.6 ± 0.9 Hz.

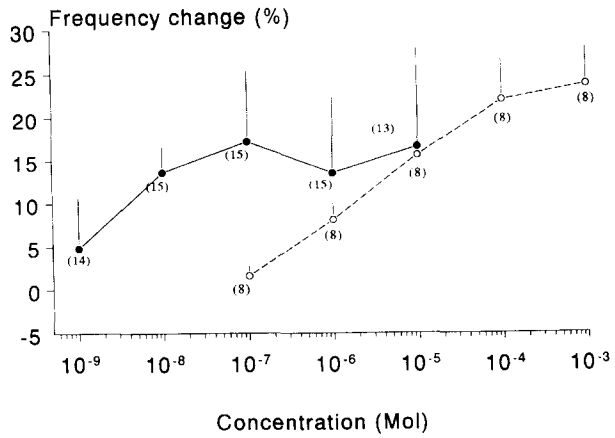

Fig. 1. The concentration-effect curve of formoterol (•) and of terbutaline (○) on ciliary beat frequency in guinea pig tracheal preparations. The effect of formoterol was significant for concentrations of 10^{-8} M or higher. Figures in brackets denote the number of explants examined at each concentration.

Of the two purified enantiomers, the S,S-enantiomer exerted no stimulatory effect on ciliary beat frequency, whereas the R,R-enantiomer stimulated ciliary beat frequency (Table 1). The maximum increase in ciliary beat frequency, $12.5 \pm 2.2\%$ was seen at a concentration of 10^{-6} M of the R,R-enantiomer.

3.2. In vivo experiments

Injections of formoterol increased mucociliary activity in the rabbit maxillary sinus in a dose-dependent manner. The log-dose response curve for this compound is shown in Fig. 2. The maximum increase, $23.0 \pm 4.0\%$, was seen after challenge with 1 nmol/kg formoterol. The dose-response curve had an inverted U-shape, with doses higher than 1 nmol/kg producing

Table 1
Effects of the R,R- and S,S-enantiomers on ciliary beat frequency of guinea pig tracheal explant preparations

Concentration	Ciliary beat frequency		% change	n	P
	Baseline	After 15 min			
Effects of the R,R-enantiomer					
10^{-8} M	8.8 ± 0.3	9.2 ± 0.3	$4.9 \pm 2.3\%$	14	NS
10^{-7} M	8.7 ± 0.3	9.5 ± 0.3	$10.4 \pm 2.0\%$	13	NS
10^{-6} M	8.7 ± 0.3	9.8 ± 0.3	$12.5 \pm 2.2\%$	13	< 0.05
Effects of the S,S-enantiomer					
10^{-8} M	8.8 ± 0.3	8.8 ± 0.3	$-0.5 \pm 1.4\%$	12	NS
10^{-7} M	8.9 ± 0.3	8.8 ± 0.4	$-1.7 \pm 2.0\%$	12	NS
10^{-6} M	9.1 ± 0.3	9.0 ± 0.3	$0.0 \pm 3.0\%$	11	NS

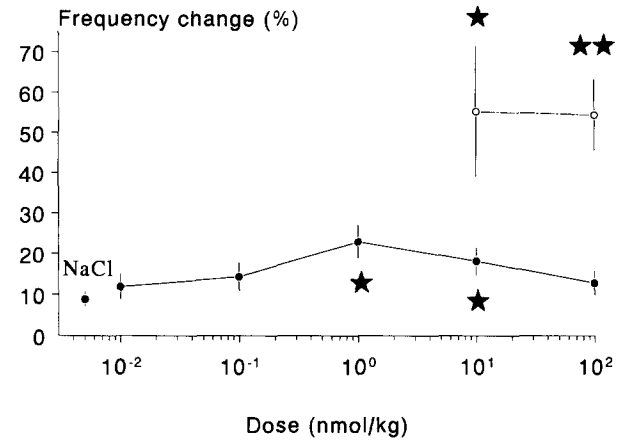

Fig. 2. The effect of increasing doses of formoterol on mucociliary activity in the rabbit maxillary sinus in vivo at dosages of 0.01–100 nmol/kg, $n = 8$ (●). (○) Shows the results of extra experiments with the two highest doses 10 and 100 nmol/kg given with an interval of 2 h, $n = 6$. Frequency zero levels were 1243 ± 228 waves/min (●) and 833 ± 282 waves/min (○). The asterisk (*) denotes $P < 0.05$.

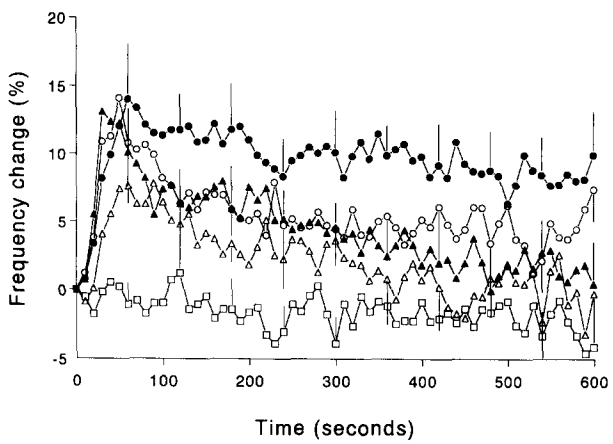

Fig. 4. The mean temporal response for the effect on mucociliary activity of 0.1 nmol/kg (○) and 1 nmol/kg (●) formoterol compared to 1 nmol/kg (△) and 10 nmol/kg (▲) terbutaline. (□) Shows the results of control experiments with the vehicle, physiological saline. $n = 7$. Only the respones during the initial 10 min are shown, as the effects of terbutaline returned to baseline levels within this period.

a weaker response. This was not due to a lingering effect of the β_2-adrenoceptor agonist, as the baseline recordings (frequency zero levels) immediately before the various challenges did not differ. However, in the extra series with 10 and 100 nmol/kg formoterol with a time lapse of 2 h between injections, the composite dose-response curve (from experimental series 1 and series 2) was sigmoidal instead. This suggests that tachyphylaxis against formoterol could have explained the inverted U-shaped curve seen in the original ascending dose-response experiments.

In the second series of experiments, the mucociliary response to challenge with formoterol was compared to the effects of terbutaline. All four doses tested (0.1 nmol/kg and 1 nmol/kg of formoterol and 1 and 10 nmol/kg of terbutaline) in this series increased the mucociliary activity (Fig. 3). The effect of the β_2-adrenoceptor agonists was noticeable already 30 s after injection (Fig. 4). There was no difference between the effects of formoterol 0.1 nmol/kg and terbutaline 1 nmol/kg, or between the effects of formoterol 1 nmol/kg and terbutaline 10 nmol/kg. However, when the two compounds were compared with regard to mucociliary response to the equimolar dose of 1 nmol/kg, formoterol increased mucociliary activity in terms of AUC significantly more than did terbutaline ($n = 7$, $P < 0.05$). After accelerating in response to a bolus dose of terbutaline, mucociliary activity returned to its baseline level within 10 min (Fig. 4), the mean duration of the effect being 7.5 ± 1.7 min at a dosage of 1 nmol/kg and 9.8 ± 2.0 min at a dosage of 10 nmol/kg. At corresponding (i.e., equi-effective) dosages, the mean duration of the effect of formoterol at 0.1 nmol/kg was 10.2 ± 2.4 min and at 1 nmol/kg 21.0 ± 3.3 min (range 10.7–30 min), the latter duration being significantly longer than that of terbutaline at either of the two dosages tested ($n = 7$, $P < 0.05$). The mucociliary response was unaffected by the order of injection (i.e., whether formoterol of terbutaline was given first).

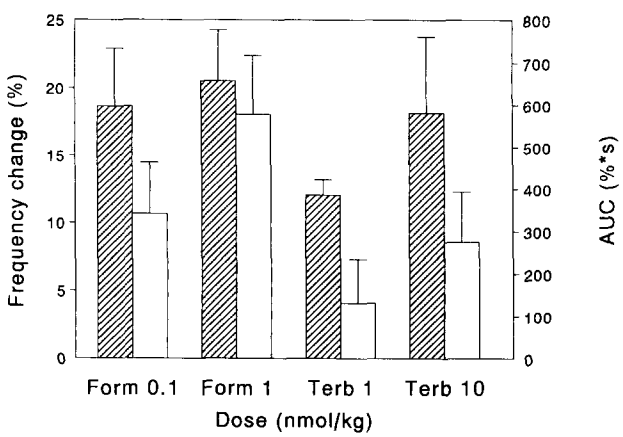

Fig. 3. The effect of formoterol (Form) and terbutaline (Terb) on mucociliary activity. Maximum responses (hatched bars, left Y-axis) and AUC (open bars, right Y-axis) calculated during the 10 min period following challenge with a bolus dose in seven rabbits. Frequency zero levels were 1009 ± 188 waves/min in the formoterol experiments and 987 ± 191 waves/min in the terbutaline experiments.

4. Discussion

The present findings show that the long-acting β_2-adrenoceptor agonist formoterol stimulates ciliary beat frequency in vitro as well as mucociliary activity in vivo.

This finding was anticipated since β_2-adrenoceptor agonists are known to stimulate mucociliary activity in the rabbit maxillary sinus (Hybbinette and Mercke, 1982a) and in the canine trachea (Wong et al., 1988b). The effect of formoterol was moderate, the increase being about 20% both in vitro and in vivo, and similar to the in vitro effect of another β_2-agonist, terbutaline. The maximum increase in vivo, approximately 25%, was almost identical to that of salbutamol, another short-acting β_2-agonist, which increased mucociliary activity in the rabbit maxillary sinus by $24.7 \pm 6.3\%$ (Hybbinette and Mercke, 1982a).

The active stereoisomer of formoterol was the R;R-enantiomer of the drug. In a study of the effect of the four different isomers of formoterol (*R,R*, *S,S*, *R,S* and *S,R*) on isolated muscles from the guinea pig trachea, the *R,R*-enantiomer was found to be much more potent than the other stereoisomers (Trofast et al., 1991). This indicates that, in the racemic mixture of which formoterol is constituted, the component responsible for the β_2-adrenoceptor stimulant effects of the drug in the airways is the *R,R*-enantiomer. However, the effect of the purified *R,R*-enantiomer was not stronger than that of the racemic mixture, in terms of either bronchial muscle activity or ciliary beat frequency.

The second series of experiments was based on the in vitro observation that formoterol on the basis of ciliary beat frequency in the guinea pig trachea, was approximately 100 times more potent than terbutaline, and it was therefore considered necessary to compare doses of the two drugs that were equi-effective rather than equimolar. The results of the in vivo comparison were consistent with the previous in vitro observation that formoterol is more potent than terbutaline, although the difference was less pronounced. It is possible that direct contact between the β_2-adrenoceptor agonist and the epithelium, as in the case of in vitro experiments, is necessary in order to reveal the full difference in potency. This view is supported by a study in asthmatic subjects, where an inhaled single dose of 12 μg formoterol was found to have roughly the same effect as 500 μg terbutaline on lung function tests including carbon monoxide diffusing capacity and gas distribution (Hedenström et al., 1992). Although the results of the present investigation were less clear-cut, the peak response and AUC for the doses of 0.1 nmol/kg formoterol and 10 nmol/kg terbutaline were approximately the same (Fig. 3), suggesting that the two compounds also differ in potency in vivo. Whether differences in the effects on the mucociliary system between formoterol and terbutaline are more pronounced after aerosol challenge is not known.

There was no difference between formoterol and terbutaline in their peak effects during a 10 min period (Fig. 3). However, measured as the time elapsed until the baseline level was reached again, the duration of the effect differed significantly between the two compounds. The duration of approximately 20 min for 1 nmol/kg formoterol after i.a. injection can be compared to the duration of the bronchodilator effect of aerosolized formoterol in conscious guinea pigs (10 min for formoterol versus 1 min for salbutamol, another common β_2-adrenoceptor stimulant in clinical use but not investigated in the present study) (Ida, 1976). The longer duration of the effects of formoterol in vivo suggests that patients with impaired mucociliary function, e.g. in asthma (Mezey et al., 1978) or chronic sinusitis (Sakakura et al., 1983), may benefit from the use of formoterol not only because of its bronchodilating effects, but also because of its effects on ciliary function in the lower and upper airways. Whether the longer duration of formoterol action also improves mucociliary clearance is not known. Clinical trials with formoterol concerning mucociliary activity and transport would therefore be of considerable interest.

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