Salmeterol, a novel, long-acting β_2 -adrenoceptor agonist: characterization of pharmacological activity *in vitro* and *in vivo*

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1 Salmeterol, a novel, long-acting β -adrenoceptor agonist, has been compared with isoprenaline and salbutamol for activity *in vitro* on a range of β -adrenoceptor containing preparations from laboratory animals and man, and *in vivo* for bronchodilator activity in the conscious guinea-pig.

2 Salmeterol, like isoprenaline and salbutamol, relaxed preparations of both guinea-pig trachea (contracted by prostaglandin (PG)F_{2 α} or electrical stimulation) and human bronchus (contracted by PGF_{2 α}) in a concentration-related fashion. Salmeterol was of similar potency to isoprenaline and more potent than salbutamol on both airway preparations.

3 Relaxant responses of superfused guinea-pig trachea and human bronchus to isoprenaline and salbutamol declined rapidly when the agonists were washed from the tissues, with complete recovery within 10 min, whereas responses to salmeterol were more persistent. In electrically-stimulated guinea-pig trachea preparations, inhibition by salmeterol persisted for periods of up to 12 h, despite continuous superfusion with agonist-free medium. However, these persistent responses were rapidly and fully reversed by the β -adrenoceptor blocking drug, propranolol (0.1 μ M). In further studies, on guinea-pig trachea, propranolol caused concentration-related parallel, rightward shifts of salmeterol concentration-effect curves, yielding a pA₂ of 9.0. The slope of the Schild plot was 1.02.

4 Another β -adrenoceptor blocking drug, sotalol (10 μ M), also fully and rapidly reversed established submaximal responses to salmeterol in superfused guinea-pig trachea. However, after administration of sotalol was stopped, the antagonism waned, and salmeterol responses were reasserted without the addition of further agonist.

5 In the β_1 -adrenoceptor containing preparation, rat left atria, isoprenaline exhibited potent, concentration-related, positive inotropic activity, whereas salbutamol and salmeterol were at least 2000–5000 fold less potent, and appeared to be partial agonists. At a concentration of 72 μ M, salmeterol exhibited weak antagonism of isoprenaline-induced increases in atrial force of contraction. This antagonism was less marked than that caused by salbutamol (42 μ M).

6 On the guinea-pig isolated gastric fundus strip, a putative β_3 -adrenoceptor containing preparation, salbutamol and salmeterol had only modest agonist activity, being 20–30 fold less potent than isoprenaline and the selective β_3 -adrenoceptor agonist, BRL 35135.

7 In conscious guinea-pigs, inhaled salmeterol and salbutamol were approximately equipotent in causing dose-related bronchodilatation. Whereas the duration of action of salbutamol at its threshold-effective dose was less than 90 min, the responses to a similarly effective dose of salmeterol were well-maintained for at least 6 h.

8 Salmeterol is therefore a potent and selective β_2 -adrenoceptor agonist with a remarkably long duration of action in isolated superfused airways smooth muscle. It also causes persistent bronchodilatation *in vivo*, in the guinea-pig, when administered by the inhaled route.

Keywords: β -Adrenoceptor agonists; salmeterol; guinea-pig trachea; human bronchus; duration of action; conscious guinea-pig; bronchodilatation

Introduction

In the treatment of bronchial asthma, inhaled β_2 -adrenoceptor agonists, such as salbutamol, produce effective bronchodilatation with few associated side-effects (McFadden, 1980; Brittain *et al.*, 1981; Clarke, 1982; Tattersfield, 1982; Svedmyr & Lofdahl, 1987). The major disadvantage of the currently available drugs is in their relatively short duration of action of 4–6 h (Leifer & Wittig, 1975; Svedmyr, 1985a,b). A bronchodilator with a substantially longer duration of action would not only have the advantage of less frequent dosing, but may afford protection against nocturnal asthma, which is currently inadequately controlled. The availability of such a compound could therefore be an important therapeutic advance. Whilst orally-administered, sustained-release preparations, pro-drugs and drugs with long plasma half-lives may offer some benefit, they lack the functional selectivity afforded by the inhaled route of administration. We have therefore attempted to design a molecule with a long duration of action at

β_2 -adrenoceptors and, which, as a consequence, would result in a long-lasting bronchodilatation after administration by inhalation.

We now describe some results comparing potency, duration of action and selectivity of salmeterol (4-hydroxy- α [[[6-[4-phenylbutyl]oxy]hexyl]amino]methyl] - 1,3 - benzenedimethanol), a novel long-acting β_2 -adrenoceptor agonist (Bradshaw *et al.*, 1987), with the β -adrenoceptor agonists, isoprenaline and salbutamol (Figure 1) both on a range of β -adrenoceptor containing preparations *in vitro*, and in conscious guinea-pigs *in vivo*.

Preliminary accounts of this work have been communicated to the British Pharmacological Society (Ball *et al.*, 1987a,b).

Methods

Tissue preparations

Tracheae and stomach fundi were obtained from male guinea-pigs (Dunkin-Hartley, 350–500 g), and atria from male rats (A

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Figure 1 Chemical structures of isoprenaline, salbutamol and salmeterol.

& H strain, 200–300 g). All animals were killed by a blow to the head and subsequent exsanguination. Human bronchi were dissected from macroscopically normal lung parenchyma, obtained from patients undergoing surgical removal of carcinomas of the lung and bronchi. Tracheal and bronchial preparations were cut into rings which were opened by cutting the cartilage opposite the smooth muscle (Coburn & Tomita, 1973). A cotton thread was tied to one cartilage for attachment to a strain gauge, and a cotton loop to the other end for anchoring the tissue in the superfusion chamber or organ bath.

Left atrial preparations were prepared as described by Blinks & Koch-Weser (1961). A thread was sewn to the uppermost portion of the atrium, and the tissue was then impaled on a 2-pin electrode and placed in a 2 ml organ bath.

The gastric fundus was dissected away from the rest of the stomach, the mucosal layer removed, and strips prepared as previously described (Coleman, 1987). One end of each preparation was attached to a strain gauge with cotton thread and the other anchored in the organ bath by means of a cotton loop.

Tissue superfusion

Superfusion apparatus The superfusion apparatus employed in these experiments has been previously described (Coleman *et al.*, 1986; Coleman & Nials, 1989). Preparations were mounted under a resting tension of 1 g, and superfused at a rate of 2 ml min^{-1} with oxygenated (5% CO_2 in O_2) modified Krebs solution maintained at 37°C . For experiments involving electrical stimulation, bipolar platinum electrodes positioned parallel with, and in close proximity to, the superfused tissue were used.

Determination of agonist activity on guinea-pig trachea and human bronchus In order to measure relaxant activity on guinea-pig trachea and human bronchus, smooth muscle tone was elevated by the inclusion of $\text{PGF}_{2\alpha}$ ($2.8 \mu\text{M}$) in the Krebs solution. Alternatively, in the case of guinea-pig trachea, phasic contractile responses were induced by electrical field-stimulation with 10 s trains of square wave pulses of 5 Hz frequency, 0.1 ms duration and just maximal voltage every 2 min. These electrically induced responses were highly reproducible over periods of at least 8–10 h of stimulation. For experiments involving electrical stimulation, preparations were taken from guinea-pigs pretreated intraperitoneally with 6-hydroxydopamine (6-OHDA, 200 mg kg^{-1}), in order to eliminate any adrenergic component in the electrically-induced response (Coleman & Nials, 1986; 1989).

For each preparation, constant responses to the standard agonist, isoprenaline, were first obtained either by repeating cumulative bolus dose-effect curves or by infusing increasing concentrations in sequential manner, until sensitivity was con-

stant. A 30 min equilibration period was then allowed before a concentration-effect curve to a test compound was started. The magnitude of each response was measured and calculated as a percentage of the maximum isoprenaline response obtained in the final control curve.

Potency values for the β -adrenoceptor agonists were expressed in both absolute terms (concentration required to induce 50% of the maximum response to each agonist, EC_{50}) and relative to isoprenaline, as an equipotent concentration (EPC, i.e. EC_{50} for the test agonist/ EC_{50} for isoprenaline). An EPC less than unity indicates greater potency than isoprenaline, and an EPC greater than unity, a lower potency than isoprenaline.

Determination of the rates of onset and offset of action Onset time (Ot_{50}) is defined as the time from administration of an EC_{50} concentration of an agonist to attainment of 50% maximal response and recovery (Rt_{50}) is defined as the time from stopping administration of the test agonist to attainment of 50% recovery from an EC_{50} concentration. Ot_{50} and Rt_{50} values were determined by interpolation from a plot of % response against time to attainment of 50% of each response (for Ot_{50}), or of 50% recovery from the response for (Rt_{50}) (Coleman & Nials, 1989).

Determination of agonist and antagonist activity in rat left atria

Atrial preparations were placed in 2 ml jacketed organ baths containing oxygenated (5% CO_2 in O_2) Krebs solution maintained at 32°C . A resting tension of 1 g was applied. Electrical stimuli of 1 Hz frequency, 1 ms duration and 5 volts amplitude were delivered by a square wave stimulator. Agonist potencies were determined in the same manner as on guinea-pig trachea and human bronchus. Responses were measured as increase in force, and expressed as a % of the maximum isoprenaline response. If the maximum increase in force achieved by the test compound was less than 50% of the preceding isoprenaline control, then it was tested for antagonist activity by repeating the isoprenaline concentration-effect curve in its presence. The isoprenaline concentration ratio was defined as:-

$$\frac{\text{EC}_{50} \text{ for isoprenaline in the presence of the test compound}}{\text{EC}_{50} \text{ for isoprenaline in the absence of the test compound}}$$

Determination of agonist activity in guinea-pig fundus

Guinea-pig fundic strip preparations were mounted in 2 ml jacketed organ baths and immersed in oxygenated (5% CO_2 in O_2) Krebs solution containing indomethacin ($2.8 \mu\text{M}$), atropine ($0.4 \mu\text{M}$) and phenoxybenzamine ($1 \mu\text{M}$), and maintained at 37°C . A tension of 1 g was applied to the tissue. Smooth muscle tone was elevated by addition of a submaximally-effective concentration of $\text{PGF}_{2\alpha}$ ($2.8 \mu\text{M}$) to the organ bath.

Agonist potencies were determined as for the other preparations, agonist potency being expressed as equipotent concentration ratio (EPC, isoprenaline = 1).

Conscious guinea-pigs

Bronchodilator activity was assessed in conscious guinea-pigs by the method described by Ball *et al.* (1987b). Briefly, guinea-pigs were placed in perspex, whole-body plethysmographs, pressure changes being recorded via a side-arm attached to a UP2 low pressure transducer. Each plethysmograph was connected via a head chamber to an aerosol expansion chamber. A gated port allowed the guinea-pig to breathe the atmosphere from the expansion chamber, but when this was closed, two other ports opened, one to a supply of 5% CO_2 in O_2 , and the other to waste. The synchronous opening and closing of the three ports was achieved with a sliding valve. Respiratory rate was continuously computed from the cyclic pressure changes in each plethysmograph. With the animals breathing the O_2/CO_2 mixture, an aerosol generated from a

9 mm histamine solution was driven into the expansion chamber for 15–30 s by use of a Devilbiss 645 nebulizer. The port to the expansion chamber was then opened and the animals exposed to the aerosolised histamine. This caused a mild bronchoconstrictor response, seen as a small fall in plethysmograph pressure and an increase in respiratory rate. The animals were exposed to histamine for 4 min or until the respiratory rate had increased by 40%, whichever occurred first, at which point they were permitted to breathe the O₂/CO₂ mixture again. During histamine exposure, a purpose-built computer measured and recorded the area under the respiratory rate curves as an index of bronchoconstriction. In most animals, repeated histamine challenges yielded reproducible bronchoconstrictor responses. Each was then treated with a β -adrenoceptor agonist or vehicle, by either the inhaled or oral route, and then re-challenged with aerosolized histamine 30, 90, 180 and 360 min later. Inhalation of β -adrenoceptor agonists was achieved by attaching each plethysmograph to a head/expansion chamber and a Devilbiss nebulizer. Guinea-pigs were exposed to aerosolized β -adrenoceptor agonists for 60 s.

Drugs used

In the present study, the following compounds were used: ascorbic acid (BDH Chemicals, U.K.), atropine sulphate (Sigma, U.S.A.), BRL35135 (Glaxo Group Research, U.K.), 5-hydroxytryptamine creatinine sulphate (5-HT, Sigma, U.S.A.), histamine acid phosphate (BDH Chemicals, U.K.), indomethacin (Sigma, U.S.A.), (–)-isoprenaline sulphate (Sigma, U.S.A.), prostaglandin F_{2α} tromethamine salt (Lutalyse, Upjohn, U.S.A.), propranolol hydrochloride (Sigma, U.S.A.), salbutamol (Glaxo Group Research, U.K.), salmeterol (Glaxo Group Research, U.K.) and sotalol hydrochloride (Glaxo Group Research, U.K.).

BRL35135, isoprenaline, salbutamol and salmeterol were each dissolved in 2 drops of glacial acetic acid and diluted to stock concentration with phosphate buffer (pH 7.0). Dilutions of stock concentrations were made with 0.9% saline. All solutions of β -adrenoceptor agonists contained ascorbic acid (11 μ M).

Indomethacin was dissolved in 1% sodium bicarbonate in 0.9% saline. Atropine, 5-hydroxytryptamine, histamine, propranolol and sotalol were dissolved in distilled water. Prostaglandin F_{2α} was supplied in aqueous solution containing 5 mg ml^{–1} free acid. All dilutions were made in 0.9% saline.

The Krebs solution used had the following composition (g l^{–1}): NaCl 6.92, NaHCO₃ 2.1, KCl 0.35, MgSO₄ · 7H₂O 0.15, KH₂PO₄ 0.16, glucose 2.0 and CaCl₂ · 6H₂O 0.28.

Results

Guinea-pig trachea

Prostaglandin F_{2α}-contracted preparation Each of the β -adrenoceptor agonists caused concentration-related relaxation of PGF_{2α}-contracted preparations of superfused guinea-pig

trachea (Figure 2, Table 1). Salmeterol and isoprenaline (EC₅₀ = 3.2 nM) were equipotent, salbutamol being approximately 2 fold less potent. All three agonists caused virtually complete inhibition of PGF_{2α}-induced tone at the highest concentrations used. However, there were differences in their rates of onset and offset of action (see Table 1). Thus, while the onset of action of both isoprenaline and salbutamol was rapid (Ot₅₀ = 1.2 and 1.8 min respectively), that for salmeterol was substantially longer (Ot₅₀ = 19.5 min). Similarly, both isoprenaline and salbutamol were short-acting (Rt₅₀ = 2.1 min and 3.5 min respectively), whereas little or no recovery was observed from responses to salmeterol for up to 45 min, the maximum time for which measurements could reliably be made.

Electrically-stimulated preparation All three β -adrenoceptor agonists caused concentration-related inhibition of electrically-induced contractile responses of the guinea-pig superfused trachea (Table 2). Salmeterol was the most potent, being approximately 2 fold more potent than isoprenaline (EC₅₀ = 15.4 nM) and approximately 8 fold more potent than salbutamol. No attempt was made in these experiments to achieve a maximal inhibitory response with either salbutamol or salmeterol (see Methods). The onset and offset of action times observed broadly reflected those on the PGF_{2α}-contracted preparation (see Table 2), with isoprenaline and salbutamol being rapid both in onset and offset of action, and salmeterol having a slow onset of action and showing virtually no recovery even after periods in excess of 12 h,

Figure 2 Prostaglandin F_{2α} (PGF_{2α})-contracted, superfused guinea-pig tracheal strip: mean relaxant concentration-effect curves for isoprenaline (O, *n* = 12), salbutamol (●, *n* = 7) and salmeterol (Δ, *n* = 5). Each point is the mean of *n* determinations, and vertical bars represent s.e.mean.

Table 1 Prostaglandin F_{2α} (PGF_{2α})-contracted superfused guinea-pig tracheal strip: potency and times for onset and recovery for isoprenaline, salbutamol and salmeterol

Agonist	Equipotent ¹ concentration (Isoprenaline = 1)	Onset ² time (Ot ₅₀ , min)	Recovery ² time (Rt ₅₀ , min)	<i>n</i>
Isoprenaline	1 EC ₅₀ = 3.2 (2.5–3.9) nM	1.2 (1.0–1.4)	2.1 (1.8–2.6)	20
Salbutamol	2.0 (1.2–3.3)	1.8 (1.3–2.5)	3.5 (2.5–4.7)	7
Salmeterol	0.9 (0.6–1.2)	19.5 (8.4–30.6)	> 12.4–> 46	12

¹ Potency data are geometric means of *n* observations with (95% CL).

² Onset and recovery data are arithmetic means of *n* observations with (95% CL) or expressed as a range.

Table 2 Electrically-stimulated guinea-pig trachea: potency and times for onset and recovery for isoprenaline, salbutamol and salmeterol

Agonist	Equipotent ¹ concentration (Isoprenaline = 1)	Onset ² time (Ot ₅₀ , min)	Recovery ² time (Rt ₅₀ , min)	n
Isoprenaline	1 EC ₅₀ = 15.4 (12.4–19.1) nM	> 1 < 3	> 2 < 4	25
Salbutamol	3.0 (1.5–6.5)	3.2 (2.0–5.4)	10.7 (7.8–14.7)	8
Salmeterol	0.4 (0.2–0.7)	29.1 (23.8–35.7)	> 420–> 770	14

¹ Potency data are geometric means of *n* observations with (95% CL).
² Onset and recovery data are arithmetic means of *n* observations with (95% CL) or expressed as a range.

despite continuous superfusion of the tissue (Figure 3). The persistent inhibitory effects of salmeterol were, however, reversed (Figure 3) by the β -adrenoceptor blocking drug propranolol (0.1 μ M).

Human bronchus

Isoprenaline, salbutamol and salmeterol all caused concentration-related relaxations of the PGF_{2 α} -contracted superfused human bronchial strip preparation (Figure 4). The mean EC₅₀ (95% confidence limits) for isoprenaline was 6.0 (3.0–9.1, *n* = 9) nM. Salmeterol was of similar potency to isoprenaline (EPC = 0.7 (0.4–1.2, *n* = 6)), while salbutamol was approximately 10 fold less potent (EPC = 10.9 (9.8–12.1, *n* = 6)). The maximum degree of relaxation induced by salmeterol was however, consistently less (70%) than that induced by isoprenaline (Figure 4), which fully relaxed the induced tone. In contrast, despite its relatively lower potency, salbutamol appeared to be a full agonist with respect to isoprenaline. Both isoprenaline (Rt₅₀ = 1.1 (0.8–1.6, *n* = 9) min) and salbutamol (Rt₅₀ = 5.5 (2.8–10.8, *n* = 6) min) were short-acting on

Figure 4 Prostaglandin F_{2 α} (PGF_{2 α})-contracted superfused human bronchial strip. Mean relaxant concentration-effect curves to isoprenaline ($\circ$, *n* = 9), salbutamol ($\bullet$, *n* = 6) and salmeterol (Δ , *n* = 6). Each point is the mean of *n* determinations, and vertical bars represent s.e.mean.

the human bronchus preparation when compared with salmeterol, the effects of which persisted with no obvious recovery

Figure 3 Electrically-stimulated, superfused, guinea-pig tracheal strip: typical inhibitory response to salmeterol, illustrating lack of recovery from inhibition despite 7 h continuous superfusion in drug-free Krebs solution, but rapid reversal by propranolol.

Figure 5 Superfused, electrically-stimulated guinea-pig trachea: representative experiment illustrating the effects of administration and subsequent washout of sotalol on the inhibitory responses persisting after washout of salmeterol. Note the reassertion of the salmeterol response without further agonist administration following washout of the sotalol.

for periods in excess of 50 min, despite continuous superfusion of the tissue.

Antagonist potency of propranolol against salmeterol on guinea-pig trachea

The interaction between propranolol and salmeterol was determined on PGF_{2α}-contracted guinea-pig tracheal preparations. Propranolol (0.1, 0.3 and 1.0 μM) caused concentration-related, parallel, rightward shifts of salmeterol concentration-effect curves, with no evidence for a reduction

in the maximal response. A pA₂ value for propranolol (mean; 95% confidence limits) of 8.97 (8.12–9.81, *n* = 11), the plot having a slope of –1.02 (0.57–1.47) was determined by the method of Arunlakshana & Schild (1959). There was, however, a marked degree of variability observed as indicated by the wide confidence limits on both pA₂ and slope.

Interaction of sotalol with salmeterol

On both PGF_{2α}-contracted and electrically-stimulated guinea-pig trachea preparations, infusions of the β-adrenoceptor blocking drug, sotalol (10 μM), caused a rapid and complete reversal of the responses persisting after administration and subsequent washout of salmeterol in concentrations (0.7–1.2 μM) sufficient to cause 75–90% maximal relaxation. When the sotalol infusion was halted, the salmeterol-induced relaxation was gradually re-established over a period of 40–60 min, despite the fact that no further salmeterol was administered (Figure 5).

In 3 experiments, administration of sotalol, and its subsequent withdrawal was repeated up to 4 times, with re-establishment of the original salmeterol-induced response on each occasion. In all of these experiments, concurrent control preparations demonstrated both the reproducibility of electrically-induced contractile responses in the absence of salmeterol and sotalol treatment over the time course of the experiment, and the maintenance of the established salmeterol-induced inhibition over the same time course.

Figure 6 Conscious guinea-pig: time course of inhibition of histamine-induced increases in respiratory rate following aerosol administration of vehicle (○) or (a) salmeterol, nebulizer concentrations 0.012 mM (■), 0.12 (▲), 1.2 (●) and 12 mM (▼) or (b) salbutamol 0.02 mM (■), 0.2 mM (▲) and 2.0 mM (●). Each point is the mean of 6 determinations. Vertical bars represent s.e. means.

Rat atria

Isoprenaline (0.4–500 nM) induced concentration-related increases in the force of contraction on rat atria, with a mean EC₅₀ value of 33 (15–71, *n* = 8) nM. The mean maximal increase in force of atrial contraction was 0.51 (0.39–0.66, *n* = 8) g. In contrast, salbutamol and salmeterol caused increases in the force of contraction only at concentrations of 1 μM and above, causing no more than 38% of the isoprenaline maximum response. When concentration-effect curves to isoprenaline were constructed in the presence of salmeterol (72 μM), a 3.5 (1.7–7.4, *n* = 5) fold, rightward displacement of the isoprenaline concentration-effect curves was observed. In contrast, the effects of salbutamol (42 μM) were more marked, producing a 68 (22–208, *n* = 6) fold rightward shift of curves to isoprenaline, indicating that the compound has some degree of affinity for β₁-adrenoceptors.

In a subsequent series of experiments, similar results were obtained with guinea-pig left atria.

Guinea-pig fundus

Isoprenaline (0.001–30 μM), salbutamol (0.01–300 μM), salmeterol (0.001–300 μM) and the selective ' β_3 -adrenoceptor agonist', BRL 35135 (0.001–30 μM), all caused concentration-related relaxation of PGF_{2 α} -contracted guinea-pig fundic strip preparations. None of the agonists was potent, isoprenaline having an EC₅₀ of 120 (90–170, $n = 48$) nM, and BRL 35135 was of similar potency, with an EPC of 1.3 (0.9–1.8, $n = 5$). Both salbutamol and salmeterol were less potent, with EPC values of 37 (35–56, $n = 5$) and 23 (4–127, $n = 4$) respectively.

The rates of relaxation of guinea-pig fundus in response to isoprenaline and salbutamol were rapid, with equilibrium being reached within 3–6 min. Salmeterol and BRL35135 caused much slower relaxations, with equilibrium being attained only after 15–30 min. No attempt was made to quantify the rate of offset of action of any of the agonists on guinea-pig fundus.

Conscious guinea-pig

Aerosol administration Inhaled nebulized solutions of salmeterol (0.012–12 mM, $n = 6$) and salbutamol (0.02–2 mM, $n = 6$) caused dose-related protection against histamine-induced bronchoconstriction in the conscious guinea-pig (Figure 6), achieving almost complete inhibition at the highest dose tested. Over the effective dose-range, there were clear differences in the durations of action of the two compounds. Thus, while threshold-effective doses (i.e. the lowest dose required to cause at least a 50% inhibition) of salbutamol (nebulizer concentration, 0.2 mM) had a duration of action of less than 1.5 h, an equi-effective dose of salmeterol (nebulizer concentration, 0.12 mM) caused an inhibition of bronchoconstriction which was well-maintained for at least 6 h.

Even when the nebulizer concentration of salbutamol was increased 10 fold, the resulting inhibition of histamine-induced bronchoconstriction lasted for less than 3 h.

Discussion

In the *in vitro* pharmacological studies, salmeterol, like isoprenaline and salbutamol, was potent in relaxing both guinea-pig and human airways smooth muscle. Its effects on guinea-pig trachea were competitively antagonized by propranolol, indicating that these effects were mediated by β -adrenoceptors. Like salbutamol, salmeterol was shown to be only a weak partial agonist on the β_1 -adrenoceptor-containing preparations, rat and guinea-pig left atria. It also possessed only modest agonist activity on the putative β_3 -adrenoceptor (Zaagsma & Nahorski, 1990)-containing preparation, guinea-pig fundus (Coleman *et al.*, 1987), where it was approximately 20 fold less potent than either isoprenaline or the selective β_3 -adrenoceptor agonist, BRL35135 (Wilson *et al.*, 1984). Although salmeterol does exhibit some relaxant activity on guinea-pig fundus, it is important to appreciate that this preparation is not sensitive to the relaxant effects of any of the β -adrenoceptor agonists, effective concentrations of salmeterol being in the micromolar range. In view of the high concentrations of salmeterol required on guinea-pig fundus and the lack of availability of a definitive β_3 -adrenoceptor blocking drug, it is by no means certain that the activity of salmeterol on this preparation is mediated by β_3 -adrenoceptors. Thus, while we can say that it is at least 20 fold weaker than isoprenaline at these receptors, it may in fact be weaker still. What is clear, however, is that salmeterol appears to possess a high degree of selectivity for β_2 -adrenoceptors.

In isolated airways smooth muscle, salmeterol has a slow onset of action, the responses in many experiments often taking periods in excess of an hour to achieve equilibrium.

This contrasts with the relatively rapid onset observed with the other β -adrenoceptor agonists included in this study. The most outstanding feature of salmeterol, however, is its long duration of action. Thus, in superfused preparations of both guinea-pig trachea and human bronchus, which demonstrated rapid and full recovery from the relaxant effects of isoprenaline and salbutamol, responses to salmeterol were well-maintained, despite continuous superfusion with drug-free medium. Indeed it was demonstrated in guinea-pig trachea that such responses were maintained for several hours. Interestingly, these responses to salmeterol were fully and rapidly reversed by infusions of the β -adrenoceptor antagonists propranolol and sotalol.

Furthermore, in guinea-pig trachea preparations in which salmeterol responses had been inhibited by sotalol, after stopping the sotalol infusion, the salmeterol-induced responses were gradually fully reasserted. The rapid and complete reversal of the sustained agonist effects of salmeterol by the β -adrenoceptor blocking drugs suggests that the long duration of its action does not result from irreversible binding to the β_2 -adrenoceptors. However, the fact that on washout of the β -adrenoceptor blocking drug, sotalol, the salmeterol effect is reasserted, suggests that the drug must persist in the tissue in such a manner that it can freely associate with and dissociate from the β -adrenoceptors. The demonstration that this process can be repeated up to four times in succession with no loss in the salmeterol response, gives further weight to this suggestion. It is interesting however, that on guinea-pig trachea at least, there appears to be a simple competitive interaction between salmeterol and propranolol which is perhaps surprising if indeed the agonist is anchored in the vicinity of the β -adrenoceptors. We have previously suggested that the mechanism by which salmeterol exhibits its long duration of action involves binding to a specific exo-site in the cell membrane adjacent to the β -adrenoceptor (Bradshaw *et al.*, 1987), and may result in part from the lipid solubility of the compound. Indeed, the rank order of both onset and offset action for the three agonists included in this study correspond with the rank order of the lipophilicities of the molecules. Clearly, data obtained from only three compounds are insufficient to be able to draw any firm conclusions and further studies are required to establish the true relationship between onset and duration of action and the differential lipid solubility of β -adrenoceptor agonists.

Salmeterol is not only a potent, long-acting β_2 -adrenoceptor agonist on airways smooth muscle preparations *in vitro*, but the present study shows that it is also a long-acting bronchodilator *in vivo* in conscious guinea-pigs. Thus, by aerosol administration, salmeterol caused dose-related inhibition of the increases in respiratory rate resulting from histamine-induced bronchoconstriction, being of a similar order of potency to salbutamol. However, as predicted from its *in vitro* activity, it exhibits a substantially longer duration of action than salbutamol. For example, at doses causing a 50% or greater inhibition of histamine-induced bronchoconstrictor responses, the effects of salmeterol persisted for at least 6 h with little or no decline in activity. In contrast, similarly effective doses of salbutamol exhibited only relatively transient activity, with no residual bronchodilatation being apparent 90 min after inhalation of a dose causing 70% inhibition of the histamine response. Even after administration of a dose of salbutamol 10 fold greater, the duration of bronchodilatation was still less than 3 h.

In clinical studies, salmeterol has also been shown to exhibit long-lasting bronchodilator activity after aerosol administration to both normal and asthmatic subjects (Maconochie *et al.*, 1987; Ullman & Svedmyr, 1988). Thus, the guinea-pig appears to resemble man, where again salmeterol is about ten fold more potent than salbutamol (Ullman & Svedmyr, 1988). Similarly, in terms of absolute duration of action, inhaled salbutamol has a well-established 4 to 6 h action in man (Leifer & Wittig, 1975), whereas preliminary data with salmeterol suggests a 12 h duration of action

(Ullman & Svedmyr, 1988). On this basis, therefore, it appears that the conscious guinea-pig is of some predictive value, in terms of relative potencies and durations of action of inhaled β_2 -adrenoceptor bronchodilators in man.

In conclusion, salmeterol is a potent and selective β_2 -adrenoceptor agonist with a long duration of action in relaxing airways smooth muscle *in vitro*. It also behaves as a

long-acting bronchodilator *in vivo*, after administration by the inhaled route to guinea-pigs, where it is substantially longer acting than salbutamol. This profile of activity parallels that previously reported in man (Maconochie *et al.*, 1987; Ullman & Svedmyr, 1988). Salmeterol may therefore represent an important advance in the treatment and management of bronchial asthma.

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