Estimation of the efficacy and affinity of the β_2 -adrenoceptor agonist salmeterol in guinea-pig trachea

I.G. Dougall, D. Harper, D.M. Jackson & P. Leff

Department of Pharmacology, Fisons plc, Bakewell Road, Loughborough, Leicestershire LE11 0RH

1 We have attempted to estimate the affinity and efficacy of the new β_2 -adrenoceptor agonist salmeterol in the guinea-pig isolated trachea and to compare these estimates with those obtained for salbutamol.

2 The kinetics of the relaxation to salmeterol were considerably slower than those to salbutamol and the responses were poorly defined. Nevertheless, in experiments employing a cumulative curve design both salmeterol and salbutamol exhibited partial agonist behaviour. Operational model-fitting of these data indicated that the intrinsic efficacies of the two agonists were indistinguishable but that salmeterol had an affinity ($pK_A = 7.4$) some 30 fold greater than salbutamol ($pK_A = 5.9$).

3 Single dose experiments, using maximally effective concentrations of salmeterol or salbutamol were carried out in an attempt to quantify more reliably the relative efficacies of the two agonists. In these experiments salbutamol was found to have an efficacy (2.5) approximately 3 fold greater than that of salmeterol (0.8).

4 Full/partial agonist interaction studies were carried out to provide another estimate of the affinity of salmeterol. Isoprenaline and adrenaline were employed as full agonists in these experiments and the affinity (pK_B) estimates obtained for salmeterol against these agonists were 7.3 and 7.4 respectively. These affinity estimates were similar to the pK_A value (7.4) obtained by operational model-fitting.

5 Salmeterol has slightly lower efficacy (3 fold) but markedly greater affinity (30 fold) than salbutamol at β_2 -receptors. The slightly lower efficacy of salmeterol is unlikely to have clinical implications for this drug as a bronchodilator β_2 -adrenoceptor agonist in asthma.

Keywords: Salmeterol; salbutamol; affinity; efficacy, guinea-pig trachea

Introduction

Salmeterol (4-hydroxy- α [[[6-[4-(phenylbutyl)oxy]-hexyl]amino]methyl]-1,3-benzene dimethanol) is a new β_2 -adrenoceptor agonist developed for the treatment of asthma. It has been shown to be both potent and long acting *in vitro* (Bradshaw *et al.*, 1987; Ball *et al.*, 1987a) and *in vivo* (Ball *et al.*, 1987b). Much emphasis has been placed on its long duration of action, and the mechanism by which it achieves such duration has been extensively investigated (Bradshaw *et al.*, 1987; Nials & Coleman, 1988; Coleman *et al.*, 1990). In contrast, the pharmacological characterization of its agonism in terms of affinity and efficacy has not been addressed, despite the obvious importance of these drug-receptor parameters in its therapeutic action.

The purpose of this study was therefore to characterize quantitatively the agonist action of salmeterol in a functional β_2 -receptor system. The results of this study are discussed with regard to the therapeutic action of salmeterol.

Methods

Guinea-pig isolated trachea

Male albino Dunkin-Hartley guinea-pigs (400–600 g) were killed by a blow to the back of the head and the whole trachea removed. After removing the adherent connective tissue, the trachea was cut into six ring segments each three cartilage bands wide and then suspended in 20 ml organ baths containing Krebs solution of the following composition (mM): NaCl 117.56, KCl 5.36, NaH_2PO_4 1.15, MgSO_4 1.18, glucose 11.10, NaHCO_3 25.00 and CaCl_2 2.55. This was maintained at 37°C and continually gassed with 5% CO_2 in O_2 . Indomethacin (2.8 μM), cocaine (30 μM), corticosterone (10 μM), ascorbate (1 mM), CGP20712A (1 μM) and phentolamine (3 μM) were added to the Krebs solution: indomethacin to prevent devel-

opment of smooth muscle tone due to the synthesis of cyclo-oxygenase products, cocaine and corticosterone to inhibit uptake₁ and uptake₂ processes respectively, ascorbate to prevent catecholamine oxidation and CGP20712A and phentolamine to avoid any complicating effects of β_1 - and α -adrenoceptor activation respectively.

The tracheal rings were suspended between two wire hooks (tungsten or stainless steel) one attached to an Ormed Beam isometric force transducer and the other to a stationary support in the organ bath. Changes in isometric force were recorded on 2-channel Advance Bryans flat bed recorders.

Experimental protocols

General At the beginning of each experiment a force of 1.0 g was applied to the tissues and this was reinstated over a 60 min equilibration period until it remained steady. Tissues were then exposed to 100 μM of the muscarinic agonist, methacholine, to assess tissue viability. All subsequent contractions were with methacholine and relaxant agonist concentration-effect, $E/[A]$, curves were constructed by cumulative (except for single dose studies, see below) additions of isoprenaline, salbutamol or salmeterol at 0.5 $\log_{10}$ unit increments. In all experiments a paired curve design was used. Responses were recorded as percentage relaxations of the methacholine-induced contraction.

Estimation of the relative efficacies of salbutamol and salmeterol

Cumulative curve design Each tissue was contracted with 3 μM methacholine and when a steady response was obtained an isoprenaline $E/[A]$ curve constructed. After washing, tissues were left for 60 min and then re-contracted with 3 μM methacholine and a second $E/[A]$ curve performed to either isoprenaline, salbutamol or salmeterol.

Single dose design This method was as for the cumulative curve design except that instead of performing a second $E/[A]$

¹ Author for correspondence.

curve, a single, maximally effective concentration of either isoprenaline (10 μ M), salbutamol (100 μ M) or salmeterol (10 μ M) was added.

Full/partial agonist interactions: estimation of affinity of salmeterol

Each tissue was contracted with 10 μ M methacholine and when a steady response was obtained an isoprenaline or adrenaline E/[A] curve constructed. After washing, tissues were left for 60 min and then re-contracted with 10 μ M methacholine. Subsequently, tissues were incubated with salmeterol or vehicle for 60 min before construction of a second isoprenaline or adrenaline E/[A] curve.

Drugs

The following drugs were used: methacholine chloride (Sigma Chemical Company), indomethacin (Sigma Chemical Company), cocaine hydrochloride (May & Baker), corticosterone 21-acetate (Sigma Chemical Company), ascorbic acid (Fisons Scientific Apparatus), phentolamine mesylate (Ciba-Geigy Laboratories), CGP20712A (ICI Laboratories), ($\pm$)-isoprenaline hydrochloride (Sigma Chemical Company), ($\pm$)-adrenaline hydrochloride (Sigma Chemical Company), ($\pm$)-salbutamol hemisulphate (Sigma Chemical Company) and salmeterol free base (Glaxo Laboratories).

Indomethacin was dissolved in 10% w/v Na₂CO₃, corticosterone 21-acetate in ethanol and isoprenaline and adrenaline in 100 μ M ascorbate. Salmeterol was made up as a stock solution of 2 mM in distilled water with the addition of a few drops of dilute hydrochloric acid. All other drugs were dissolved in distilled water.

Data analysis

Operational model-fitting In order to estimate agonist efficacies and affinities experimental E/[A] curve data were fitted using the operational model of agonism (Black & Leff, 1983; Black *et al.*, 1985):

$$E = \frac{E_m \tau^n [A]^n}{(K_A + [A])^n + \tau^n [A]^n} \quad (1)$$

in which E and [A] are the pharmacological effect and the concentration of the agonist, respectively; E_m is the maximum possible effect; K_A is the agonist dissociation constant (this was estimated as the negative logarithm, that is pK_A); τ is the efficacy of the agonist (estimated as a logarithm); n determines the steepness of the occupancy-effect relation.

In the case of the cumulative curve design the partial agonist (salbutamol or salmeterol) curve data were analysed according to the comparative method (Barlow *et al.*, 1967) by fitting them to the operational model (equation 1) simultaneously to fitting the full agonist (isoprenaline) data to a logistic function of the form (Leff *et al.*, 1989):

$$E = \frac{E_m [A]^n}{[A_{50}]^n + [A]^n} \quad (2)$$

where E_m and n are as defined above and $[A_{50}]$ is the location parameter for the full agonist curve. (Location parameters were estimated as negative logarithms ($p[A_{50}]$)). This allows K_A and τ for the partial agonist to be estimated as well as E_m and n from each pair of curves.

In the case of the single dose experiments, each individual isoprenaline E/[A] curve was fitted to equation 2 to give estimates of E_m and n. These values were used to estimate the corresponding τ values using the following relationship, which is the operational model definition of the intrinsic activity, α , of the agonist (Black *et al.*, 1985):

$$\frac{\alpha}{E_m} = \frac{\tau^n}{1 + \tau^n} \quad (3)$$

where E_m , τ and n are as defined above and α is the response produced by the single dose of partial agonist. It is not possible to estimate the affinity of the partial agonists from such experiments.

Logistic curve fitting In control experiments designed to determine any time-dependent changes in tissue sensitivity and in full/partial interaction experiments, individual E/[A] curves were fitted to a logistic of the form:

$$E = \frac{\alpha [A]^m}{[A_{50}]^m + [A]^m} \quad (4)$$

where α , $[A_{50}]$ and m are the asymptote (or intrinsic activity), location and slope parameters respectively.

Full/partial agonist interaction analysis

Dose-ratios (r) were calculated from the $[A_{50}]$ estimates for each pair of curves and fitted to equation 5 (Arunlakshana & Schild, 1959). The $[A_{50}]$ of the full agonist curve in the presence of the partial agonist was estimated as the concentration that produced half the maximum increment that could be obtained. (This maximum increment was the difference between the response to the partial agonist alone and the maximum response of which the tissue was capable).

$$\log_{10}(r - 1) = n \log_{10}[B] + pK_B \quad (5)$$

If n, the Schild slope parameter, was not significantly different from unity it was constrained to this value in order to estimate pK_B ($-\log_{10} K_B$).

All data fitting procedures were carried out using the statistical package, BMDP, and a Vax 11/780 mainframe computer. Differences were assessed by Student's *t* test and considered significant at the level of $P < 0.05$.

Results

Estimation of the relative efficacies of salbutamol and salmeterol

Figure 1 shows the relaxant effects of isoprenaline, salbutamol and salmeterol on tracheal rings contracted with 3 μ M methacholine. The kinetics of the relaxations were similar for isoprenaline and salbutamol but those of salmeterol were considerably slower and the curves poorly defined. Indeed in many instances the curves were so poorly defined as to be unmeasurable. This was particularly noticeable at low concentrations of salmeterol, responses at high concentrations being reasonably well-defined. However, it was evident from such experiments that both salbutamol and salmeterol produced smaller maximum relaxations than isoprenaline.

The apparently partial agonist behaviour of salbutamol and salmeterol were analysed by applying the comparative method (Barlow *et al.*, 1967). Firstly, control experiments showed that paired isoprenaline E/[A] curves were not significantly different in terms of maximum (α) and slope (m). A small but significant rightward shift in the location ($p[A_{50}]$) of the 2nd curve occurred. This time-dependent change in potency was not corrected for as it would have little effect on the results. The mean logistic parameters ($\pm$ s.e.; $n = 4$) of the curves were as follows: 1st curve: $\alpha = 97.0 (\pm 0.8)$; $m = 1.3 (\pm 0.1)$; $p[A_{50}] = 7.4 (\pm 0.1)$. 2nd curve: $\alpha = 93.7 (\pm 1.5)$; $m = 1.4 (\pm 0.1)$; $p[A_{50}] = 7.2 (\pm 0.1)$.

Results of applying the comparative method to salbutamol and salmeterol are illustrated in Figure 2a and b respectively. The lines drawn through the data are the results of operational model-fitting. Analysis of 4 experiments gave average estimates for efficacy (τ) and affinity (pK_A) of 2.0 ($\log \tau = 0.3 (\pm 0.1)$) and 5.9 (± 0.1) for salbutamol and 1.3 ($\log \tau = 0.1$

Figure 1 Representative experimental recordings of isoprenaline (a), salbutamol (b) and salmeterol (c) E/[A] curves. Tissues were contracted with 3 μ M methacholine ($\blacktriangle$). Applied concentrations of agonist are given in μ M units. Note the different time scale in (c).

Figure 2 Analysis of salbutamol and salmeterol E/[A] curve data by operational model-fitting. In both (a) and (b) the data shown are from single tissues and the lines drawn through the points are the results of operational model-fitting. (a) Illustrates the analysis of salbutamol ($\bullet$) with isoprenaline ($\circ$) used as the reference full agonist. The model parameter estimates were as follows: $E_m = 90.1$; $n = 1.3$; $\tau = 1.3$; $pK_A = 6.0$; $p[A_{50}]$ (isoprenaline) = 7.2. (b) Illustrates the analysis of salmeterol ($\blacktriangle$). The model parameter estimates were as follows: $E_m = 96.2$; $n = 1.5$; $\tau = 1.1$; $pK_A = 7.4$; $p[A_{50}]$ (isoprenaline) = 7.2. See Table 1 for full results.

Table 1 Mean parameter estimates obtained from operational model-fitting of cumulative E/[A] curve data

	Salbutamol	Salmeterol
$p[A_{50}](\text{Isop})$	7.3 ± 0.1	7.3 ± 0.1
n	1.3 ± 0.1	1.4 ± 0.1
E_m	96.4 ± 3.1	98.2 ± 1.3
$\log \tau$	0.3 ± 0.1	0.1 ± 0.1
pK_A	5.9 ± 0.1	7.4 ± 0.1
Replicates	4	4

(± 0.1) and $7.4 (\pm 0.1)$ for salmeterol respectively. The efficacy estimates were not significantly different (see Table 1 for complete results).

Single dose experiments

Although the above experiments gave estimates of the efficacy and affinity of salmeterol, we felt that the slow kinetics of the salmeterol responses may have compromised them due to possible difficulties with response definition and measurement. In the time period needed to complete the E/[A] curve it was likely that the methacholine-induced contraction would have faded significantly. In fact, control experiments indicated that the contraction faded by $19\% \pm 2\%$ ($\pm$ s.e.; $n = 4$, data not shown) over the time period needed to complete a salmeterol E/[A] curve. Such fade would result in the efficacy of salmeterol being overestimated. The single dose experiments were therefore conducted to give a more reliable estimation of the efficacy of salmeterol (affinity estimates cannot be determined with this experimental design). Isoprenaline E/[A] curves were constructed in each tissue and this was followed by a single concentration of isoprenaline (10 μ M), salbutamol (100 μ M) or salmeterol (10 μ M). These concentrations were previously determined to be maximally effective (see Figure 2). The results of these experiments are shown in Figure 3. The data in Figure 3(a) indicate that there was not a time-dependent change in maximum response to isoprenaline. As was the case with the cumulative curve design, both salbutamol (Figure 3(b) and salmeterol (Figure 3(c)) produced smaller maximum relaxations than isoprenaline. Salbutamol produced on average 80% ($\pm 10\%$; $n = 6$) and salmeterol 40% ($\pm 10\%$; $n = 6$) of the maximum relaxation to isoprenaline; that is, their respective intrinsic activities were 0.8 and 0.4. Analysis of the data as described in the Methods section gave efficacy estimates of 2.5 ($\log \tau = 0.4 (\pm 0.1)$) for salbutamol and 0.8 ($\log \tau = -0.1 (\pm 0.1)$) for salmeterol (see Table 2 for complete results). These efficacy estimates were significantly different.

Full/partial agonist interactions: estimation of the affinity of salmeterol

Since it was not possible to estimate the affinity of salmeterol from the single dose experiments we adopted another approach in which the ability of salmeterol to antagonize the effects of higher efficacy agonists (isoprenaline and adrenaline) was investigated. In these experiments tracheal rings were

Table 2 Mean parameter estimates obtained from single dose experiments

	Isoprenaline	Salbutamol	Salmeterol
$p[A_{50}](\text{Isop})$	7.1 ± 0.2	7.2 ± 0.1	7.1 ± 0.1
n	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
E_m	92.1 ± 4.3	92.9 ± 2.6	92.5 ± 1.9
α	88.6 ± 1.7	69.1 ± 5.7	41.3 ± 7.6
α/E_m	1.0 ± 0.1	0.8 ± 0.1	0.4 ± 0.1
$\log \tau$	—	0.4 ± 0.1	-0.1 ± 0.1
Replicates	6	6	6

Figure 3 Effect of single doses of isoprenaline, salbutamol and salmeterol. The data are the average of 6 replicates with vertical lines indicating s.e. In each panel the $E/[A]$ curve data are for isoprenaline. The single points represent the effects produced by $10 \mu\text{M}$ isoprenaline ($\bullet$) (a), $100 \mu\text{M}$ salbutamol ($\blacktriangle$) (b) and $10 \mu\text{M}$ salmeterol ($\blacksquare$) (c). See Table 2 for model parameter estimates.

contracted with $10 \mu\text{M}$ methacholine rather than $3 \mu\text{M}$ as used previously. Under these conditions of greater functional antagonism, salmeterol produced smaller relaxations allowing better defined full agonist $E/[A]$ curves to be constructed. In this instance control experiments indicated small but significant changes in the maximum response (α) and location ($p[A_{50}]$) of consecutive isoprenaline $E/[A]$ curves. As above, these changes were considered to have minimal effect on the subsequent analyses. Adrenaline control pairs were not significantly different. The mean logistic parameters ($\pm$ s.e.; $n = 6$ (isoprenaline) or $n = 4$ (adrenaline)) were as follows: isoprenaline; 1st curve $\alpha = 77.6 (\pm 3.3)$; $m = 1.3 (\pm 0.1)$; $p[A_{50}] = 7.1 (\pm 0.1)$. 2nd curve: $\alpha = 71.4 (\pm 4.6)$; $m = 1.3 (\pm 0.1)$; $p[A_{50}] = 7.0 (\pm 0.1)$. Adrenaline: 1st curve $\alpha = 88.2 (\pm 3.6)$; $m = 1.3 (\pm 0.1)$; $p[A_{50}] = 6.0 (\pm 0.1)$. 2nd curve: $\alpha = 79.2 (\pm 4.6)$; $m = 1.3 (\pm 0.1)$; $p[A_{50}] = 6.0 (\pm 0.1)$.

Figures 4a and 5a show typical antagonist effects of $1 \mu\text{M}$ salmeterol on isoprenaline and adrenaline $E/[A]$ curves respectively. The raised baseline of the second $E/[A]$ curves indicates the amount of relaxation produced by $1 \mu\text{M}$ salmeterol in these tissues. It should be noted that $E/[A]$ curves performed in the presence of salmeterol were significantly less steep (i.e. the midpoint slope parameter, m , was smaller) than control curves. The reason for this is unclear but it indicated that the interactions did not conform with simple competition. Nevertheless, the calculated dose-ratios from several of these experiments are displayed in Schild Plot form in Figures 4b and 5b. Analysis of these curve displacements using equation (5) yielded Schild slope parameter (n) estimates of 1.2 ± 0.1 (s.e., 22 d.f.) and 1.1 ± 0.1 (s.e., 18 d.f.) for isoprenaline and adrenaline respectively. Neither value was significantly different from unity and the resulting pK_B estimates were 7.3 ± 0.1

Figure 4 Antagonism of isoprenaline by salmeterol: (a) shows $E/[A]$ curve data from a single tissue for isoprenaline in the absence of ($\circ$) and the presence of $1 \mu\text{M}$ salmeterol ($\bullet$); (b) illustrates the results of several such experiments in Schild plot form. The slope of the plot was not significantly different from unity (1.2 ± 0.1) (22 d.f.) and the estimated pK_B was 7.3 ± 0.1 (23 d.f.).

(s.e., 23 d.f.) and 7.4 ± 0.1 (s.e., 19 d.f.). These estimates were not significantly different from each other.

Discussion

The purpose of this study was to quantify the activity of the β_2 -adrenoceptor agonist, salmeterol, in terms of its affinity and efficacy and to compare it with salbutamol.

Previous studies (Ball *et al.*, 1987a,b; Bradshaw *et al.*, 1987) have shown that salmeterol has a slower onset of action than salbutamol and a longer duration of action. The slow onset

Figure 5 Antagonism of adrenaline by salmeterol: (a) shows $E/[A]$ curve data from a single tissue for adrenaline in the absence of ($\circ$) and the presence of $1 \mu\text{M}$ salmeterol ($\bullet$); (b) illustrates the results of several such experiments in Schild plot form. The slope of the plot was not significantly different from unity (1.1 ± 0.1) (18 d.f.) and the estimated pK_B was 7.4 ± 0.1 (19 d.f.).

was also evident in the present study (see Figure 1); the time course of salmeterol $E/[A]$ curves was considerably slower than those of isoprenaline and salbutamol. In many instances the responses to salmeterol were so poorly defined as to preclude accurate measurement. Even in the cases that were measurable it was impossible to assess how much of the relaxant response was due to a β_2 -mediated effect and how much was the result of time-dependent fade of the methacholine-induced contraction (control experiments indicated that the average degree of fade was 19% but this is obviously tissue-dependent). The estimates of efficacy and affinity for salmeterol obtained by operational model-fitting of the $E/[A]$ curve data must therefore be treated with some caution. Such experiments indicated that salbutamol and salmeterol had efficacies that were indistinguishable ($\tau = 2.0$ and 1.3 respectively) but that salmeterol ($pK_A = 7.4$) had approximately 30 fold higher affinity for β_2 -receptors than salbutamol ($pK_A = 5.9$). However, if the maximum response to salmeterol were overestimated as a result of a time-dependent loss of tone, as suggested by control experiments then efficacy would be overestimated.

The purpose of the 'single dose' experiments (Figure 3) was to provide a more reliable estimate of the relative efficacies of salmeterol and salbutamol. Although a maximally effective concentration of salmeterol takes significantly longer than that of salbutamol to equilibrate, any time-dependent loss of tone would be expected to be markedly less than that occurring in the course of construction of a salmeterol $E/[A]$ curve. These experiments yielded τ estimates of 2.5 for salbutamol and 0.8 for salmeterol; that is salbutamol has approximately 3 fold higher efficacy than salmeterol. In terms of intrinsic activity these efficacy estimates correspond to 0.8 and 0.4 respectively (see Table 2). Under the experimental conditions used, therefore, salmeterol was capable of generating 50% of the relaxation produced by salbutamol. The important consideration from the therapeutic point of view is whether such a difference would be relevant in terms of bronchodilator activity. In principle, partial agonist behaviour in man would be undesirable. However, the difference between salmeterol and salbutamol, at least on the scale operating in the present experiments, is of a size which is unlikely to have clinical implications.

Having obtained what we felt was a reliable estimate of the relative efficacies of salbutamol and salmeterol, we then attempted to estimate the affinity of salmeterol by performing full/partial agonist interactions. Full agonist $E/[A]$ curves obtained in the presence of salmeterol were significantly less

steep than control curves. The reason for this is unclear but it may be a consequence of the slow offset of salmeterol limiting the establishment of new equilibrium with the full agonist. Strictly speaking this observation precludes Schild analysis of the data as one of the criteria for simple competitive behaviour is violated. However, in order to obtain estimates of the affinity of salmeterol we fitted the data to equation 5 and estimated pK_B values. With isoprenaline and adrenaline as the full agonists, the pK_B values obtained were 7.3 and 7.4 respectively. These values were not significantly different and thus confirm the agonist-independence of salmeterol's antagonism.

Although these affinity estimates cannot be considered as true pK_B 's (for the reason stated above) their similarity to the pK_A estimate (7.4) obtained by operational model-fitting increases confidence in their reliability. It should also be noted that in full/partial agonist interaction experiments the affinity of the partial agonist obtained by Schild analysis is an overestimate. The overestimation is a consequence of the ability of the partial agonist to produce a response, the higher the efficacy of the partial agonist the bigger the error in the affinity estimate (Kenakin, 1987). In the case of a system in which the agonist $E/[A]$ curves are hyperbolic the affinity of a partial agonist with an intrinsic activity of 0.5 is overestimated by 0.3 $\log_{10}$ units. In the above experiments the error would be expected to be less than 0.3 $\log_{10}$ units since the intrinsic activity of salmeterol was less than 0.5.

Salmeterol and salbutamol differ only in the N-substituent on their ethanolamine side-chain, salmeterol having a long lipophilic chain and salbutamol having a tertiary butyl group. It has been suggested that the long duration of action of salmeterol results from the binding of its lipophilic side-chain to an exo-site within the β_2 -receptor but distinct from the physiological receptor (Bradshaw *et al.*, 1987). If an exo-site does exist the affinity of a compound which binds to it and to the physiological β_2 -receptor would be expected to be greater than that of structurally similar compounds which bind only to the latter. Indeed, binding studies have indicated that the affinity of salmeterol is approximately 50 fold greater than that of salbutamol (Coleman *et al.*, 1990). In contrast, such exo-site binding would be expected to have little effect on the response-generating capacity (efficacy) of the agonist. The results of this study which show that salmeterol has markedly higher affinity (approx. 30 fold) but only slightly lower efficacy (3 fold) than salbutamol lend support to these suggestions.

We wish to thank Glaxo Group Research Limited for their gift of salmeterol.

References

- ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 45–58.
- BALL, D.I., COLEMAN, R.A., DENYER, L.H., NIALS, A.T. & SHELDRIK, K.E. (1987a). *In vitro* characterization of the β_2 -adrenoceptor agonist, salmeterol. *Br. J. Pharmacol.*, **92**, 591P.
- BALL, D.I., COLEMAN, R.A., DENYER, L.H., NIALS, A.T. & SHELDRIK, K.E. (1987b). Bronchodilator activity of salmeterol, a long-acting β_2 -adrenoceptor agonist. *Br. J. Pharmacol.*, **92**, 746P.
- BARLOW, R.B., SCOTT, N.C. & STEPHENSON, R.P. (1967). The affinity and efficacy of onium salts on frog rectus abdominis. *Br. J. Pharmacol.*, **31**, 188–196.
- BLACK, J.W. & LEFF, P. (1983). Operational models of pharmacological agonism. *Proc. R. Soc. B.*, **220**, 141–162.
- BLACK, J.W., LEFF, P., SHANKLEY, N.P. & WOOD, J. (1985). An operational model of agonism: the effect of $E/[A]$ curve shape on agonist dissociation constant estimation. *Br. J. Pharmacol.*, **84**, 561–571.
- BRADSHAW, J., BRITTAIN, R.T., COLEMAN, R.A., JACK, D., KENNEDY, I., LUNTS, L.H.C. & SKIDMORE, I.F. (1987). The design of salmeterol, a long-acting selective β_2 -adrenoceptor agonist. *Br. J. Pharmacol.*, **92**, 590P.
- COLEMAN, R.A., JOHNSON, M., NIALS, A.T. & SUMNER, M.J. (1990). Salmeterol, but not formoterol, persists at β_2 -adrenoceptors *in vitro*. *Br. J. Pharmacol.*, **99**, 121P.
- KENAKIN, T.P. (1987). Drug antagonism. In *Pharmacologic Analysis of Drug-Receptor Interaction*. pp. 205–244. New York: Raven Press.
- LEFF, P., PRENTICE, D.J., GILES, H., MARTIN, G.R. & WOOD, J. (1989). Estimation of agonist affinity and efficacy by direct, operational model-fitting. *J. Pharmacol. Methods*, **23**, 225–237.
- NIALS, A.T. & COLEMAN, R.A. (1988). The interaction between salmeterol and β_2 -adrenoceptor blocking drugs on guinea-pig isolated trachea. *Br. J. Pharmacol.*, **95**, 540P.

(Received May 16, 1991)

Revised August 15, 1991

Accepted August 27, 1991)