Gen. Pharmac. Vol. 27, No. 4, pp. 575–580, 1996

ELSEVIER

ISSN 0306-3623/96 $15.00 + .00
SSDI 0306-3623(95)02052-7

REVIEW

Some Pharmacodynamic Aspects on Long-Acting β-Adrenoceptor Agonists

Bertil Waldeck

DEPARTMENT OF PHARMACOLOGY, PRECLINICAL R&D, ASTRA DRACO AB,
P.O. BOX 34, S-221 00 LUND, SWEDEN. FAX: 46-46-33-66-66

ABSTRACT. **1. Formoterol and salmeterol are the first members of a new generation of long-acting β_2-adrenoceptor agonists for inhalation. The discovery of the long effect duration of formoterol was made by chance, while the development of salmeterol appeared to follow a purposeful research strategy.**

2. Preclinical evaluation predictive of the clinical duration of effect of long-acting bronchodilators is not straightforward. Experiments *in vitro* may give false positive results, while experiments *in vivo* may show false negative results.

3. Once the principle of a long duration of effect was established, a number of novel long-acting β_2-adrenoceptor agonists of various chemical structure have emerged.

4. There are two alternative models for the explanation of the long duration of effect: the exosite binding explaining the mode of action of salmeterol, and the more general diffusion microkinetic model applicable for both formoterol and salmeterol.

5. Long-acting β-adrenoceptor agonists with a relatively low efficacy like salmeterol may, under certain circumstances, inhibit competitively the relaxing effect of agonists with higher efficacy like formoterol and salbutamol.

6. Like all other β_2-adrenoceptor agonists in current clinical use, formoterol and salmeterol comprise racemic mixtures. Only the RR- and R-enantiomers are pharmacologically active. The experimental compounds TA-2005 and picumeterol have been developed as pure RR- and R-enantiomers, respectively. GEN PHARMAC 27;4:575–580, 1996.

KEY WORDS. Formoterol, salmeterol, stereoisomers, airways

INTRODUCTION

Ever since the discovery of the relieving effect of adrenaline in asthma (Bullowa and Kaplan, 1903), synthetic derivatives of this hormone with increasingly improved therapeutic properties have been developed. The latest clinically significant achievement is a prolonged duration of action. There are today two long-acting β_2-adrenoceptor agonists for inhalation on the market, formoterol and salmeterol. Formoterol, with the laboratory code BD40A, was synthesized some 20 years ago (Murase *et al.*, 1977) as a β_2-selective adrenoceptor agonist in the wake of terbutaline and salbutamol. In the early preclinical documentation there was little that sorted out formoterol from its predecessors apart from a very high potency (Ida, 1976). It was only when Löfdahl and Svedmyr (1986) 10 years later tried inhaled formoterol in asthmatic patients that the long duration of effect was discovered. It is worth noting that the first clinical trial with salmeterol in asthma was performed by the same research team at about the same time (Ullman and Svedmyr, 1988).

While formoterol is a typical example of serendipity, salmeterol appears to be the result of a purposeful preclinical research strategy based on binding to hypothetical exosites (Bradshaw *et al.*, 1987). In fact, there is still a dispute as to which compound is the real long-acting one (Nials *et al.*, 1994a). After the discovery of formoterol and salmeterol, new candidates for long-acting β_2-adrenoceptor agonists have emerged (Fig. 1). Thus the carbostyril derivative TA-2005 (Kikkawa *et al.*, 1991) may be derived from the structure of formoterol. Picumeterol, code number GR114297A (Nials *et al.*, 1993b), has structural similarities with salmeterol. Somewhat different in molecular shape but mutually related are SOM 1122 (Grice and O'Donnell, 1987) and RP 58802B (Underwood *et al.*, 1992). Prolonged duration of action of a β-adrenoceptor agonist may be achieved also with the use of the prodrug principle (Svensson, 1987). Thus bambuterol, the bis-*N*,*N*-dimethyl-carbamate of terbutaline (Olsson and Svensson, 1984), shows a markedly prolonged duration of action after oral administration. However, prodrugs are beyond the scope of this review.

Received 20 June 1995.

PRECLINICAL EVALUATION OF THE DURATION OF EFFECT

Formoterol and salmeterol

The duration of the bronchodilating effect may be determined either *in vitro* or *in vivo*. *In vitro* experiments are valuable for screening purposes and for detailed analysis of the mechanism of action while the experimental conditions *in vivo* include kinetic and metabolic factors as well. The first preclinical estimation of the duration of effect of inhaled formoterol was made on conscious guinea-pigs challenged with histamine given as an aerosol (Ida, 1976). This study, using a simple scoring system to assess the degree of dyspnoe, did not convincingly demonstrate superiority over salbutamol.

Once the long duration of action of formoterol was demonstrated on patients, a more detailed preclinical evaluation started. In these experiments, salmeterol was usually used as a reference. It was now demonstrated in conscious guinea-pigs, with computer-aided estimation of the dyspnoe, that inhaled formoterol had a protecting effect against histamine provocation which lasted some-

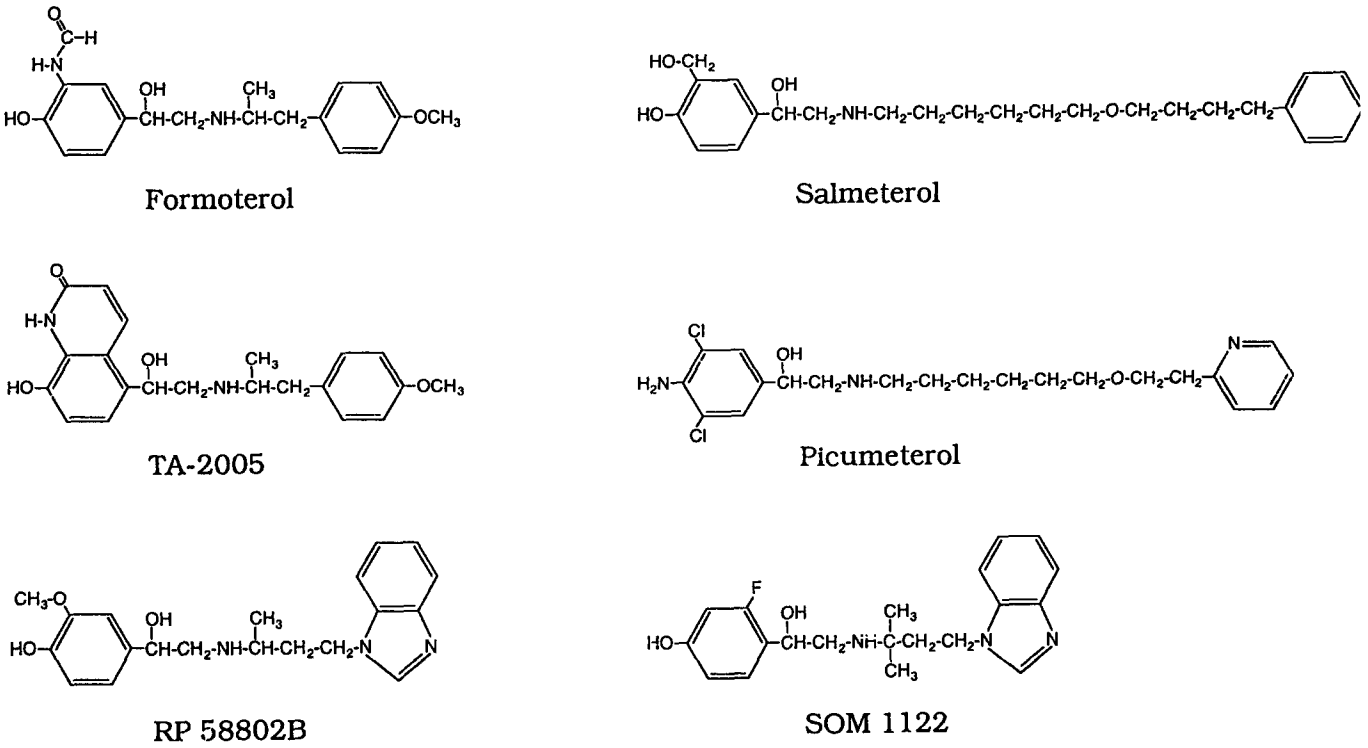

FIGURE 1. Chemical structures of some long-acting β-adrenoceptor agonists.

what longer than that seen after inhaled salbutamol, but not more than 3 hr, whereas salmeterol protected the animals for 6 hr or more (Nials *et al.*, 1994a). In asthmatic patients, formoterol and salmeterol appear to be equally long-acting (Rabe *et al.*, 1993b). Thus the classical histamine provocation test on conscious guinea-pigs, driven to collapse, may give *false negative results* in terms of duration of the bronchodilating effect.

Their ability to inhibit plasma exudation induced by asthma mediators is another property of β_2-adrenoceptor agonists. In the guinea-pig, formoterol topically applied on the tracheal mucosa, inhibited bradykinin-induced plasma exudation for 5 hr. At this time interval the protecting effect of salbutamol was gone (Erjefält and Persson, 1991). This was the first study in guinea-pigs *in vivo* showing a marked difference in the duration of effect of formoterol and salbutamol.

In isolated tracheal smooth muscle from guinea-pig, precontracted with carbachol, the relaxing effect of formoterol and salmeterol was less readily reversed by washing than that of salbutamol, but the relaxed state was reversed by the β-adrenoceptor antagonist propranolol (Jeppsson *et al.*, 1989a). Moreover, it was observed that the onset of action was considerably slower for salmeterol than for formoterol and salbutamol. D2343, an experimental β_2-adrenoceptor agonist which showed a marginally extended duration of effect in clinical trials (Löfdahl and Svedmyr, 1984), behaved more like salmeterol than formoterol in this test system. Apparently, reversal by washing in vitro may give *false positive estimates* of the duration of effect.

Also when isolated human bronchi were washed, formoterol and salmeterol gave a slower reversal than salbutamol (Naline *et al.*, 1994), although formoterol, but not salmeterol, appeared to act during a shorter period at the lower concentration range (Nials *et al.*, 1993a).

Another interesting feature of formoterol and salmeterol is the "reassertion behaviour," that is the ability to return to the relaxed state after removal of the antagonist from the medium (Lindén *et al.*, 1991; Ball *et al.*, 1991). This reassertion cycle may be repeated several-fold indicating a strong retention of the agonist in the receptor region. Since formoterol appears to show an extended duration in man only when inhaled (Löfdahl and Svedmyr, 1989), the role played by the airway epithelilum was examined (Ullman *et al.*, 1992). Removal of the epithelium from guinea-pig tracheal strip preparations had no influence, neither on the onset of action, nor on the reversal by washing, and formoterol behaved essentially as salmeterol.

Bronchodilating β-adrenoceptor agonists inhibit contractions induced by electrical field stimulation of tracheal strip preparations from guinea-pigs. Also in this experimental system, the onset of action of formoterol was fast compared with salmeterol whereas the duration of effect during continuous washing was intermediate compared with that of salbutamol and salmeterol (Nials *et al.*, 1994a). In experiments with an isolated and electrically stimulated vagus nerve-trachea tube preparation, the resorcinol derivative of salmeterol, D2489, was compared with terbutaline (Jeppsson *et al.*, 1989b). Both compounds inhibited the contractions completely. The effect of terbutaline was rapidly reversed by washing. The inhibition by D2489 resisted washing but was reversed by propranolol. It was also shown that D2489 accumulated in the tracheal tissue but terbutaline did not.

The isolated, perfused and ventilated lung offers a more integrated test system. The effect kinetics of equieffective, inhaled doses of formoterol and salmeterol was compared with that of terbutaline on the isolated guinea-pig lung during single pass perfusion. The bronchospasmolytic effect was measured as protection against acetylcholine provocations. The onset of action was immediate for formoterol and terbutaline while it was delayed for salmeterol (Jeppsson *et al.*, 1994). The protecting effect of terbutaline disappeared completely within 30 min whereas the effect of formoterol and salmeterol remained largely unchanged during 50 min of perfusion.

Taken together the available experimental evidence shows, *in vivo* and *in vitro*, that both formoterol and salmeterol are long-

acting compared with terbutaline or salbutamol. Their relative duration of effect appears to vary depending on the experimental conditions used. This variation may indicate qualitative differences in effect kinetics between formoterol and salmeterol. In spite of this limitation of predictability, a similar experimental approach has been used in the evaluation of the long-acting β_2-adrenoceptor agonists which emerge after formoterol and salmeterol.

TA-2005 and picumeterol

The first report on TA-2005 (Kikkawa *et al.*, 1991) described this compound as an extremely potent and highly selective β_2-adrenoceptor agonist but did not mention anything about the duration of effect. In a subsequent study (Voss *et al.*, 1992), it was shown that the offset of the relaxing effect of TA-2005 on isolated guinea-pig trachea was slow compared with salbutamol. This study also showed that TA-2005 binds tightly to β_2-adrenoceptors (on membranes from bovine skeletal muscle) suggesting a sustained activation. In this context, it should be recalled that Standifer *et al.* (1989) reported on two carbostyril-based β-adrenoceptor agonists, carboamine and carbo-Br. These compounds, very closely related to TA-2005, were found to bind next to irreversibly and with high affinity to the receptor. Further studies with TA-2005 on guinea-pig *in vitro* (Voss, 1994) and *in vivo* (Kikkawa *et al.*, 1994) demonstrated a duration of action comparable with that of formoterol and salmeterol. In asthmatic patients the bronchodilating effect of inhaled TA-2005 (6 or 9 μg) lasted for approximately 30 hr (Voss, 1994).

Picumeterol (GR114297A) is a new compound, structurally related to salmeterol. It has been evaluated on electrically stimulated guinea-pig trachea *in vitro* and on histamine-challenged, conscious guinea-pig *in vivo* in the same way as its parent compound (Nials *et al.*, 1993b). The duration of effect of picumeterol was found to be in between that of salbutamol and salmeterol.

RP58802B and SOM 1122

RP58802B seems to have been evaluated mainly under *in vivo* conditions (Underwood *et al.*, 1992). Thus, nebulized RP58802B produced an inhibition of histamine-induced bronchospasm of rapid onset and long duration in the anesthetized guinea-pig. In equieffective doses salbutamol was shorter- and salmeterol longer-acting than RP58802B. Furthermore, RP58802B was found to inhibit PAF-induced microvascular leakage (Underwood *et al.*, 1992) but the duration of this effect was not reported.

SOM1122 differs structurally from RP58802B in the substitution of the phenyl ring and in the branching of the side chain. Similar to salmeterol it has a slow onset of the relaxing effect on isolated tracheal smooth muscle (Grice and O'Donnell, 1987). In anesthetized guinea-pigs, inhaled SOM1122 inhibited dose-dependently acetycholine-induced bronchoconstriction in the guinea-pig (Fügner, 1989). This study also showed that SOM1122 inhibited histamine-induced extravasation and allergen-induced influx of eosinophiles in the guinea-pig lung. No statement on the duration of action of this compound was made. However, SOM1122 has been employed as a tool in psychopharmacological studies (O'Donnell, 1988).

MECHANISM OF THE LONG DURATION OF EFFECT

The long duration of action of salmeterol has been explained in terms of firm binding to exo-receptor sites in the vicinity of the β-adrenoceptors (Bradshaw *et al.*, 1987; Johnson *et al.*, 1993). The idea that the pharmacodynamic and/or the pharmacokinetic properties of a β-adrenoceptor agonist may be modified by binding to exo-sites just outside the physiological receptor was, however, presented a decade previously (Brittain *et al.*, 1976). A similar approach in the design of new β-adrenoceptor agonists has been made by another research team (Jacobson *et al.*, 1983; Rosenkranz *et al.*, 1986). By attaching a "carrier molecule" to a β-adrenoceptor agonist via a "spacer arm" the carrier may interact with sites outside the β-adrenoceptor proper while the agonist is still in touch with the adrenoceptor.

Although there is some indirect evidence (Nials *et al.*, 1993c), direct experimental support for the existence of these hypothetical exo-sites has not been published. Recently, attempts were made to cut short the duration of action of salmeterol in strips from guinea-pig trachea by preincubation with fragments of the salmeterol molecule thought to interact with the exo-sites. No change in the duration of effect of salmeterol was observed (Bergendal *et al.*, 1996).

A more general working hypothesis is the plasmalemma diffusion microkinetic theory (Anderson, 1993; Anderson *et al.*, 1994b). According to this model, the lipid bilayer of airway smooth muscle acts as a depot for lipophilic agonists. The onset of action and the duration of effect is then determined by the physiocochemical properties of the molecule. The highly lipophilic salmeterol is thought to approach the receptor mainly via migration in the lipid bilayer, hence the slow onset of action. Formoterol with an intermediate lipophilicity may approach the receptor from both the aqueous phase and the lipid bilayer, hence both rapid onset and slow offset of action. The hydrophilic and short-acting salbutamol does not pile up in the plasmalemma. In support of the diffusion microkinetic model, specific differences in biophysical properties between salbutamol, formoterol and salmeterol have been found (Anderson *et al.*, 1994a).

An interesting relationship between lipophilicity and effect kinetics for a number of β-adrenoceptor agonists was found in a series of experiments with an electrically stimulated vagus nerve-tracheal tube preparation (Jeppsson *et al.*, 1989a, 1989b). The test compounds, which inhibited the nerve-induced contractions, were added cumulatively either to the fluid-filled lumen (intratracheally) or into the external bathing medium (extratracheally). A small difference in pEC_{50} between the two modes of administration is characteristic of a compound which is retained in the smooth muscle region. Long-acting β-adrenoceptor agonists were found among compounds with a high lipid solubility and a small difference in pEC_{50} while hydrophilic compounds with a short duration of action showed a big difference (Fig. 2). However, lipophilicity per se does not guarantee a long duration of action (Jeppsson *et al.*, 1989a; Coleman *et al.*, 1994) and local metabolism may terminate the effect.

DIFFERENCES IN EFFICACY

In airway smooth muscle the concentration-response curve for a β-adrenoceptor agonist with high efficacy is shifted to the right in a parallel manner when the tone is increased. For an agonist with low efficacy the maximum relaxation is reduced. This old knowledge was illustrated by experiments with the high efficacy agonist terbutaline and the low efficacy agonist sulfonterol (Waldeck *et al.*, 1986). Moreover, when the smooth muscle tone is high the low efficacy agonist will competitively inhibit the effect of a high efficacy agonist.

Salmeterol has a relatively low efficacy compared with salbutamol while formoterol appears to be more efficacious than this standard reference compound as observed on guinea-pig trachea (Dougall *et al.*, 1991; Lemoine and Overlack, 1992; Lindén *et al.*, 1993) and on the dog saphenous vein (Nials *et al.*, 1994b). Salmeterol

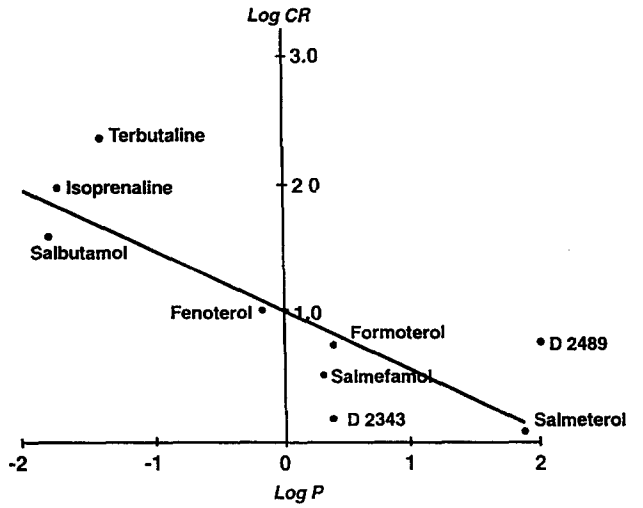

FIGURE 2. Relation between effect kinetics and lipophilicity for some β-adrenoceptor agonists. Inhibition of contraction was measured in an electrically stimulated vagus nerve-trachea tube preparation from guinea-pig. The log difference (Log CR) for pEC_{50} obtained with extra- versus intra-tracheal administration of drug was plotted against the logarithm for the octanol/water distribution-coefficient at pH 7.4 (Log P). Data from Jeppsson *et al.* (1989a, 1989b).

was found to inhibit the relaxing effect of adrenaline, isoprenaline (Dougall *et al.*, 1991) and formoterol (Jeppsson *et al.*, 1992) on the carbachol-contracted, isolated guinea-pig trachea in a competitive manner. In both studies, a linear Schild plot and a pA_2 (or pK_B) value about 7.4 was reported. Similar results were obtained with formoterol and salmeterol on human bronchus *in vitro* (Källström *et al.*, 1994). Furthermore, salmeterol antagonizes the inhibitory effect of formoterol on LTB_4-induced hydrogen peroxide generation by guinea-pig peritoneal eosinophiles (Rabe *et al.*, 1993a) and the inhibition of stimulated eosinophil peroxidase secretion caused by salbutamol in human peripheral blood eosinophils (Munoz *et al.*, 1995).

According to classical receptor theory, the degree of inhibition of an antagonist should be independent of the agonist used provided only one type of receptor is involved. It was found that the inhibition by salmeterol in guinea-pig trachea varied with the agonist used, being more pronounced against salbutamol than against fenoterol (Källström *et al.*, 1994). In contrast, the inhibition by sulfonterol appeared to follow the general rule. This may indicate that the interaction of salmeterol with β-adrenoceptors is complex.

The low efficacy and firm binding of salmeterol demonstrated under *in vitro* conditions is difficult to translate into clinical terms. Theoretically, the presence of a partial agonist may increase the dose required of an agonist with higher efficacy to obtain maximum bronchodilation in an emergency situation with severe bronchospasm. In patients with mild asthma, however, the combined effects of salmeterol and salbutamol is largely additive (Smyth *et al.*, 1993). Another theoretical consideration is that tolerance develops more slowly to a low efficacy agonist than to a high efficacy agonist. These questions have to be settled in well designed clinical experiments.

STERIC ASPECTS

All bronchodilating β-adrenoceptor agonists in current clinical use, including formoterol and salmeterol, are racemic mixtures. Formoterol which has two chiral centres is a 50/50 mixture of the RR- and SS-enantiomers. The eudismic ratio (potency ratio more active/less active enantiomer) for formoterol is about 800 as assessed on tracheal smooth muscle (Table 1). The less active SS-enantiomer does not interfere with the relaxing effect of the RR-enantiomer (Trofast *et al.*, 1991). The diastereomers of formoterol (RS- and SR-configuration), which are not included in the pharmaceutical preparation, have an intermediate effect and do not show stereoselectivity. But the relaxing effect of all enantiomers is blocked by propranolol (Trofast *et al.*, 1991).

TA-2005, which is the pure RR-enantiomer, showed a very high stereoselectivity towards its SS-enantiomer (Table 1). This is not surprising since the configuration around the chiral centres of the molecule is identical with that of formoterol. In marked contrast, salmeterol, with an unbranched side chain, has a eudismic ratio around 40, 100 times lower than that of TA-2005 (Table 1). This, together with the absence of stereoselectivity for the diastereomers of formoterol, shows that the geometry around the amino group is critical for the affinity and efficacy of a β-adrenoceptor agonist. Both enantiomers of salmeterol appear to have a long duration of action (Nials *et al.*, 1994c).

Picumeterol has been developed as the pure R-enantiomer (Nials *et al.*, 1993b), but no information on the activity of the S-enantiomer is available. The enantiomeric state of SOM1122 (Fügner, 1989) and RP58802B (Underwood *et al.*, 1992) has not been explicitly stated, but presumably only the racemates have been studied. This is a crucial point because enantiomers do not always differ in potency only, as is the case with formoterol and salmeterol, but may have quite different pharmacologic effects (Waldeck, 1993). The effects of the individual enantiomers should be elucidated before extensive studies on a racemate are commenced.

CONCLUDING REMARKS

Long-acting β_2-adrenoceptor agonists comprise a new generation of compounds added to the armamentarium of drugs used for symptomatic treatment of asthma. The pharmacology of these compounds shows that the number of chemical modifications of the natural prototype, adrenaline, leading to modification of pharmacodynamic and pharmacokinetic properties of the molecule appears to be unlimited. New members of this group of compounds, whenever available, should be thoroughly examined for new useful properties and, when appropriate, tested clinically since at the end only clinical results count.

TABLE 1. Steric aspects of long-acting β_2-adrenoceptor agonists. Potency (pEC_{50}) for the relaxing effect on guinea-pig tracheal smooth muscle and the relation (eudismic ratio) between the more active and the less active enantiomer

Enantiomer	pEC_{50}	Eudismic ratio	Source
(R;R)-formoterol	9.89		
(S;S)-formoterol	6.96	850	Trofast et al. (1991)
(R;S)-formoterol	7.87		
(S;R)-formoterol	7.83	1	Trofast et al. (1991)
(R;R)-TA-2005	9.29		
(S;S)-TA-2005	5.62	4,680	Voss et al. (1992)
(R)-salmeterol	8.77		
(S)-salmeterol	7.09	47	Johnson et al. (1993)

References

Anderson G. P. (1993) Formoterol: pharmacology, molecular basis of agonism, and mechanism of long duration of a highly potent and selective β_2-adrenoceptor agonist bronchodilator. *Life Sci.* **52**, 2145–2160.

Anderson G. A., Jäkel K., Mutz M. and John E. (1994a) Direct biophysical measurements support the diffusion microkinetic model of long acting beta-adrenoceptor agonist action. *Am. J. Resp. Crit. Care Med.* **149(4)**, A481.

Anderson G. P., Lindén A. and Rabe K. F. (1994b) Why are long-acting beta-adrenoceptor agonists long-acting? *Eur. Respir. J.* **7**, 569–578.

Ball D. I., Brittain R. T., Coleman R. A., Denyer L. H., Jack D., Johnson M., Lunts L. H. C., Nials A. T., Sheldrick K. E. and Skidmore I. F. (1991) Salmeterol, a novel, long-acting β_2-adrenoceptor agonist: characterization of pharmacological activity *in vitro* and *in vivo*. *Br. J. Pharmac.* **104**, 665–671.

Bergendal A., Lindén A., Skoogh B.-E., Gerspacher M., Anderson G. P. and Lofdahl C.-G. (1996) Salmeterol-mediated reassertion of relaxation persists in guinea-pig trachea pretreated with aliphatic side chain structural analogues. *Br. J. Pharmac.* (in press).

Bradshaw J., Brittain R. T., Coleman R. A., Jack D., Kennedy I., Lunts L. H. C. and Skidmore I. F. (1987) The design of salmeterol, a long-acting selective β_2-adrenoceptor agonist. *Br. J. Pharmac.* **92**, 590P.

Brittain R. T., Dean C. M. and Jack D. (1976) Sympathomimetic bronchodilator drugs. *Pharmac. Ther. B.* **2**, 423–462.

Bullowa J. G. M. and Kaplan D. M. (1903) On the hypodermatic use of adrenalin chloride in the treatment of asthmatic attacks. *Medical News* **83**, 787–790.

Coleman R. A., Nials A. T., Johnson M. and Vardey C. J. (1994) Salmeterol: relationship between duration of action and lipophilicity? *Am. J. Resp. Crit. Care Med.* **149(4)**, A481.

Dougall I. G., Harper D., Jackson D. M. and Leff P. (1991) Estimation of the efficacy and affinity of the β_2-adrenoceptor agonist salmeterol in guinea-pig trachea. *Br. J. Pharmac.* **104**, 1057–1061.

Erjefält I. and Persson C. G. A. (1991) Long duration and high potency of antiexudative effects of formoterol in guinea-pig tracheobronchial airways. *Am. Rev. Respir. Dis.* **144**, 788–791.

Fügner A. (1989) Formation of oedema and accumulation of eosinophils in the guinea-pig lung. *Int. Arch. Allergy Appl. Immunol.* **88**, 225–227.

Grice J. F. and O'Donnell S. R. (1987) A new β_2-selective adrenoceptor agonist, SOM 1122. *Clin. Exp. Pharmacol. Physiol.* Suppl. 10, 51.

Ida H. (1976) Cardiorespiratory activities of 3-formylamino-4-hydroxy-α-(*N*-1-methyl-2-*p*-methoxyphenethylaminomethyl)-benzylalcoholhemifumarate (BD40A) and some other β-adrenoceptor stimulants in conscious guinea pigs. *Arzneim.-Forsch.* **26**, 1337–1340.

Jacobson K. A., Marr-Leisy D., Rosenkranz R. P., Verlander M.S., Melmon K. L. and Goodman M. (1983) Conjugates of catecholamines. I. *N*-Alkyl-functionalized carboxylic acid congeners and amides related to isoproterenol. *J. Med. Chem.* **26**, 492–499.

Jeppsson A.-B., Löfdahl G., Waldeck B. and Widmark E. (1989a) On the predictive value of experiments *in vitro* in the evaluation of the effect duration of bronchodilator drugs for local administration. *Pulmonary Pharmacology* **2**, 81–85.

Jeppsson A.-B., Roos C., Waldeck B. and Widmark E. (1989b) Pharmacodynamic and pharmacokinetic aspects on the transport of bronchodilator drugs through the tracheal epithelium of the guinea-pig. *Pharmacol. Toxicol.* **64**, 58–63.

Jeppsson A.-B., Källström B.-L. and Waldeck B. (1992) Studies on the interaction between formoterol and salmeterol in guinea-pig trachea *in vitro*. *Pharmacol. Toxicol.* **71**, 272–277.

Jeppsson A.-B., Nilsson E. and Waldeck B. (1994) Formoterol and salmeterol are both long acting compared to terbutaline in the isolated perfused and ventilated guinea-pig lung. *Eur. J. Pharmac.* **257**, 137–143.

Johnson M., Butchers P. R., Coleman R. A., Nials A. T., Strong P., Sumner M. J., Vardey C. J. and Whelan C. J. (1993) The pharmacology of salmeterol. *Life Sci.* **52**, 2131–2143.

Källström B.-L., Sjöberg J. and Waldeck B. (1994) The interaction between salmeterol and β_2-adrenoceptor agonists with higher efficacy on guinea-pig trachea and human bronchus *in vitro*. *Br. J. Pharmac.* **113**, 687–692.

Kikkawa H., Naito K. and Ikezawa K. (1991) Tracheal relaxing effects and β_2-selectivity of TA-2005, a newly developed bronchodilating agent, in isolated guinea-pig tissues. *Japan J. Pharmac.* **57**, 175–185.

Kikkawa H., Kanno K. and Ikezawa K. (1994) TA-2005, a novel, long-acting, and selective β_2-adrenoceptor agonist: characterization of its *in vivo* bronchodilating action in guinea pigs and cats in comparison with other β_2-agonists. *Biol. Pharm. Bull.* **17**, 1047–1052.

Lemoine H. and Overlack C. (1992) Highly potent beta-2 sympathomimetics convert to less potent partial agonists as relaxants of guinea-pig tracheae maximally contracted by carbachol: comparison of relaxation with receptor binding and adenylate cyclase stimulation. *J. Pharmac. Exp. Ther.* **261**, 258–270.

Lindén A., Bergendal A., Ullman A., Skoogh B.-E. and Löfdahl C.-G. (1991) High concentration of formoterol and salmeterol in the isolated guinea-pig trachea: reassertion of smooth muscle relaxation after beta blockade followed by wash-out. *Am. Rev. Respir. Dis.* **143**, 4(2), A749.

Lindén A., Bergendal A., Ullman A., Skoogh B.-E. and Löfdahl C.-G. (1993) Salmeterol, formoterol, and salbutamol in the isolated guinea-pig trachea: differences in maximum relaxant effect and potency but not in functional antagonism. *Thorax* **48**, 547–553.

Löfdahl C.-G. and Svedmyr N. (1984) A new β_2-adrenoceptor agonist—D2343—with long duration. Inhalation comparison with terbutaline in asthmatic patients. *Allergy* **39**, 485–489.

Löfdahl C.-G. and Svedmyr N. (1986) Effect duration of inhaled formoterol, a new β_2-adrenoceptor agonist, compared to salbutamol in asthmatic patients. *Acta Pharmacol. Toxicol.* Suppl. **5(2)**, 229.

Löfdahl C.-G. and Svedmyr N. (1989) Formoterol fumarate, a new β_2-adrenoceptor agonist. *Allergy* **44**, 264–271.

Munoz N. M., Rabe K. F., Vita A. J., McAllister K., Mayer D., Weiss M. and Leff A. R. (1995) Paradoxical blockade of beta adrenergically mediated inhibition of stimulated eosinophil secretion by salmeterol. *J. Pharmac. Exp. Ther.* **273**, 850–854.

Murase K., Mase T., Ida H. Takahashi K. and Murakami M. (1977) New β-adrenoceptor stimulants. Studies on 3-acylamino-4-hydroxy-α-(*N*-substituted aminomethyl)benzyl alcohol. *Chem. Pharm. Bull.* **25**, 1368–1377.

Naline E., Zhangy Y., Qian Y., Mairon N., Anderson G. P., Grandordy B. and Advenier C. (1994) Relaxant effects and durations of action of formoterol and salmeterol on the isolated human bronchus. *Eur. J. Pharmac.* **7**, 914–920.

Nials A. T., Coleman R. A., Johnson M., Magnussen H., Rabe K. F. and Vardey C. J. (1993a) Effects of β-adrenoceptor agonists in human bronchial smooth muscle. *Br. J. Pharmac.* **110**, 1112–1116.

Nials A. T., Coleman R. A., Johnson M. and Vardey C. J. (1993b) GR114297A: a potent and long-acting β_2-adrenoceptor agonist. *Eur. Resp. J.* **6**, Suppl. 17, P1882.

Nials A. T., Sumner M. J., Johnson M. and Coleman R. A. (1993c) Investigations into factors determining the duration of action of the β_2-adrenoceptor agonist salmeterol. *Br. J. Pharmac.* **108**, 507–515.

Nials A. T., Ball D. I., Butchers P. R., Coleman R. A., Humbles A. A., Johnson M. and Vardey C. J. (1994a) Formoterol on airway smooth muscle and human lung mast cells: a comparison with salbutamol and salmeterol. *Eur. J. Pharmac.* **251**, 127–135.

Nials A. T., Coleman R. A., Johnson M. and Vardey C. J. (1994b) Determination of intrinsic activities of a range of β_2-adrenoceptor agonist bronchodilators. *Am. J. Resp. Crit. Care Med.* **149**(4), A481.

Nials A. T., Coleman R. A., Johnson M. and Vardey C. J. (1994c) The β-adrenoceptor pharmacology of the enantiomers of salmeterol. *Am. J. Resp. Crit. Care Med.* **149**(4), A481.

O'Donnell J. M. (1988) Psychopharmacological consequences of activation of beta adrenergic receptors by SOM-1122. *J. Pharmac. Exp. Ther.* **246**, 38–46.

Olsson O. A. T. and Svensson L.-Å. (1984) New lipophilic terbutaline ester prodrugs with long effect duration. *Pharm. Res.* **1**, 19–23.

Rabe K. F., Giembycz M. A., Dent G., Perkins R. S., Evans P. and Barnes P. J. (1993a) Salmeterol is a competitive antagonist at β-adrenoceptors mediating inhibition of respiratory burst in guinea-pig eosinophils. *Eur. J. Pharmac.* **231**, 305–308.

Rabe K. F., Jörres R., Nowak D., Behr N. and Magnussen H. (1993b) Comparison of the effects of salmeterol and formoterol on airway tone and responsiveness over 24 hours in bronchial asthma. *Am. Rev. Respir. Dis.* **147**, 1436–1441.

Rosenkranz R. P., Jacobson K. A., Verlander M. S., Klevans L., O'Donnell M., Goodman M. and Melmon K. L. (1986) Conjugates of catecholamines. IV. *In vitro* and *in vivo* pharmacological activity of monodisperse oligopeptide conjugates. *J. Pharmac. Exp. Ther.* **227**, 267–273.

Smyth E. T., Pavord I. D., Wong C. S., Wisniewski A. F. Z., Williams J. and Tattersfield A. E. (1993) Interaction and dose equivalence of salbutamol and salmeterol in patients with asthma. *Br. Med. J.* **306**, 543–545.

Standifer K. M., Pitha J. and Baker S. P. (1989) Carbostyril-based beta-adrenergic agonists: evidence for long lasting or apparent irreversible receptor binding and activation of adenylate cyclase activity *in vitro*. *Naunyn Schmiedeberg's Arch. Pharmacol.* **339**, 129–137.

Svensson L.-Å. (1987) Bambuterol—a prodrug-prodrug with built-in hydrolysis brake. *Acta Pharm. Suec.* **24**, 333–341.

Trofast, J., Österberg K. Källström B.-L. and Waldeck B. (1991) Steric aspects of agonism and antagonism at β-adrenoceptors: synthesis of and pharmacological experiments with the enantiomers of formoterol and their diastereomers. *Chirality* **3**, 443–450.

Ullman A. and Svedmyr N. (1988) Salmeterol, a new long-acting inhaled β_2-agonist: comparison with salbutamol in adult asthmatic patients. *Thorax* **43**, 674–678.

Ullman A., Bergendahl A., Lindén A., Waldeck B., Skoogh B.-E. and Löfdahl C.-G. (1992) Onset of action and duration of effect of formoterol and salmeterol compared to salbutamol in isolated guinea-pig trachea with or without epithelium. *Allergy* **47**, 384–387.

Underwood S. L., Lewis S. A. and Raeburn D. (1992) RP58802B, a long-acting β_2-adrenoceptor agonist: assessment of antiasthma activity in the guinea-pig *in vivo*. *Pulmonary Pharmacology* **5**, 203–212.

Voss H.-P. (1994) Long-acting β_2-adrenoceptor agonists in asthma: molecular pharmacological aspects. *Thesis*, Vrije Univeriteit, Amsterdam.

Voss H.-P., Donnell D. and Bast A. (1992) Atypical molecular pharmacology of a new long-acting β_2-adrenoceptor agonist, TA-2005. *Eur. J. Pharmac.* **227**, 403–409.

Waldeck B., Jeppsson A.-B. and Widmark E. (1986) Partial agonism and functional selectivity: a study on β-adrenoceptor mediated effects in tracheal, cardiac and skeletal muscle. *Acta Pharmacol. Et Toxicol.* **58**, 209–218.

Waldeck B. (1993) Biological significance of the enantiomeric purity of drugs. *Chirality* **5**, 350–355.