ABSTRACT. 1. Formoterol and salmeterol are the first members of a new generation of long-acting \( \beta \)-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 \( \beta \)-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 \( \beta \)-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 \( \beta \)-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.

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 \( \beta \)-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 \( \beta \)-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 Lofdahl 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 \( \beta \)-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 58820B (Underwood et al., 1992). Prolonged duration of action of a \( \beta \)-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.

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 dyspnoea, 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 dyspnoea, that inhaled formoterol had a protecting effect against histamine provocation which lasted some-
what longer than that seen after inhaled salbutamol, but not more than 3 hr, whereas salmeterol protected the animals for 6 hr or more (Nals 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 β₂-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 β₂-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 (Nals 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 epithelium 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 (Nals 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-

![Chemical structures](image-url)

**FIGURE 1.** Chemical structures of some long-acting β-adrenoceptor agonists.
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 β₂-adrenoceptor agonists which emerge after formoterol and salmeterol.

**TA-2005 and picumetol**

The first report on TA-2005 (Kikkawa et al., 1991) described this compound as an extremely potent and highly selective β₂-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 β₂-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 carboxyl-based β₂-adrenoceptor agonists, carbo-amine 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).

Picumetol (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 picumetol was found to be in between that of salbutamol and salmeterol.

**RP58802B and SOM 1122**

RP58802B seems to have been evaluated mainly under in vitro 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 acetylcholine-induced bronchoconstriction in the guinea-pig (Fugner, 1989). This study also showed that SOM1122 inhibited histamine-induced extravasation and allergen-induced influx of eosinophils 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 physicochemical 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 pEC50 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 pEC50 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 dose 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 sul福特eral (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 (Dougal et al., 1991; Lemoine and Overlack, 1992; Lindén et al., 1993) and on the dog saphenous vein (Nials et al., 1994b). Salmeterol...
was found to inhibit the relaxing effect of adrenaline, isoprenaline (Jeppsson et al., 1989a, 1989b).

FIGURE 2. Relation between effect kinetics and lipophilicity of drugs. The log difference (Log CR) for pEC50 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).

...erol 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 β2-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 β2-adrenoceptor agonists. Potency (pEC50) for the relaxing effect on guinea-pig tracheal smooth muscle and the relation (eudismic ratio) between the more active and the less active enantiomer

<table>
<thead>
<tr>
<th>Enantiomer</th>
<th>pEC50</th>
<th>Eudismic ratio</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R,R)-formoterol</td>
<td>9.89</td>
<td>850</td>
<td>Trofast et al. (1991)</td>
</tr>
<tr>
<td>(S,S)-formoterol</td>
<td>6.96</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>(R,S)-formoterol</td>
<td>7.87</td>
<td>1</td>
<td>Trofast et al. (1991)</td>
</tr>
<tr>
<td>(S,R)-formoterol</td>
<td>7.83</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>(S,S)-TA-2005</td>
<td>5.62</td>
<td>4,680</td>
<td></td>
</tr>
<tr>
<td>(R)-salmeterol</td>
<td>8.77</td>
<td>47</td>
<td>Johnson et al. (1993)</td>
</tr>
<tr>
<td>(S)-salmeterol</td>
<td>7.09</td>
<td>47</td>
<td></td>
</tr>
</tbody>
</table>

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).
Pharmacodynamic Aspects of Long-Acting $\beta$-Agonists

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