

Enantiomers of bronchodilating β_2 -adrenoceptor agonists: Is there a cause for concern?

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All bronchodilating β_2 -adrenoceptor agonists in current clinical use are derivatives of adrenaline and are available as racemates. Whereas the vast majority of the pharmacologic and clinical documentation has been made with the racemates, there are a few studies with the individual enantiomers. It thus appears that all established pharmacologic effects of racemic β_2 -agonists reside in the (R)-enantiomer, with the (S)-enantiomer being virtually inactive. In recent years the suspicion has been raised that the (S)-enantiomer of the β_2 -agonists is responsible for induction of airway hyperreactivity. This suspicion is based primarily on results obtained in guinea pigs exposed to (S)-enantiomers of β_2 -agonists. A number of experiments *in vitro* have been undertaken to find a mechanism of action for these observations *in vivo*. Most of the results obtained are equivocal. However, the observation that (S)-salbutamol may cause mobilization of intracellular Ca^{2+} , apparently by means of a cholinergic mechanism, deserves further investigation. The clinical studies are focused on the enantiomers of salbutamol. They confirm the preclinical findings that the pulmonary, as well as the extrapulmonary, effects of salbutamol reside in the (R)-enantiomer. The studies available so far do not convincingly show clinically significant airway hyperreactivity after exposure to the (S)-enantiomer. Further studies are needed to settle this issue. (J Allergy Clin Immunol 1999;103:742-8.)

Key words: Bronchodilator, β -adrenoceptor agonist, asthma, airway smooth muscle, hyperreactivity, enantiomer, racemate

All bronchodilating β -adrenoceptor agonists in current clinical use are derivatives of adrenaline and are available as racemates. Whereas the natural prototype comprises the pure (R)-enantiomer, large-scale stereoselective production of the synthetic congeners has, until recently, not been feasible. Accordingly, the vast majority of the comprehensive pharmacologic and clinical documentation of this class of compounds has been made with the racemates. However, a few studies have been performed with the pure enantiomers prepared on a laboratory scale. In general, the pharmacologic activity of derivatives of phenylethanamine related to adrenaline resides in the (–)-(R)-enantiomer, with the (+)-(S)-enantiomer being virtually inactive.¹

Abbreviation used

COPD: Chronic obstructive pulmonary disease

There is substantial evidence that *rac*- β -adrenoceptor agonists during some experimental conditions may cause airway hyperreactivity to various contractile stimuli in animals, as well as in human subjects.²⁻⁶ This phenomenon has in part been interpreted as secondary to desensitization of β -adrenoceptors. In recent years, the suspicion has been raised that the distomer, usually the (+)-(S)-enantiomer, of the β -agonist is responsible for induction of airway hyperreactivity and may contribute to increased asthma morbidity and mortality.^{7,8} The aim of this survey is to critically review the collected evidence for and against this hypothesis. To give a complete picture of the subject, the stereoselectivity of β -adrenoceptor agonists in general, both their dynamic and kinetic aspects, and the significance of the enantiomeric purity will be discussed.

ON NOMENCLATURE AND ENANTIOMERIC PURITY

The nomenclature for the enantiomers of phenylethanamines in the literature is not uniform. In early papers the optical activity was generally noted as (–)- and (+)- for the levo- and dextro-rotatory enantiomers. When the absolute configuration became established, the designation (R)- or (S)- was used according to Cahn et al.⁹ In the following, the designation (R)- and (S)- is used rather than (–)- and (+)-, even if the absolute configuration has not been explicitly stated in the original report. This is in conformity with the conditions for the endogenous compounds, adrenaline and noradrenaline.¹ For compounds with 2 chiral centers, the configuration at both positions is given (ie, [R;R]-formoterol).¹⁰ In one of the articles cited, there is an inappropriate use of the descriptors D- and L-, where D-salbutamol probably refers to (R)-salbutamol.¹¹ The potency ratio between the more active enantiomer (the *eutomer*) and the less active one (the *distomer*) is referred to as the *eudismic ratio*.

In the pharmacologic literature the enantiomeric purity of chiral drugs is seldom specified. Because resolution into enantiomers is often far from complete, this means that the pharmacologic profile displayed by the distomer may, to a variable degree, be modified by the presence of the eutomer.¹² In fact, it is generally impossible to judge whether the residual effect of the distomer of a β -agonist

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is due to traces of the eutomer or whether it is an inherent effect. Without specification of the enantiomeric purity, statements on eudismic ratios may be more or less meaningless. To avoid misinterpretation of data obtained with a distomer, control experiments with the eutomer in concentrations or doses corresponding to the degree of contamination must be performed.

STEREOSELECTIVITY OF BRONCHODILATING β -ADRENOCEPTOR AGONISTS

Effects on airway smooth muscle and cilia

For salbutamol and terbutaline, it was shown, soon after their discovery, that their bronchodilating capacity, measured as relaxation of isolated smooth muscle from guinea pig trachea, resides predominantly in the (R)-enantiomer.^{13,14} With salbutamol, it was further shown that this stereoselectivity extends to in vivo conditions, with inhibition of acetylcholine-induced bronchoconstriction as an effect parameter.¹⁵ In a subsequent study with highly purified enantiomers of terbutaline, a 1000-fold potency difference between the two was demonstrated.¹⁶ It was also shown that the relaxing effect of (R)-terbutaline on the guinea pig trachea was not inhibited by the presence of an excessive concentration of (S)-terbutaline.¹⁶ Similar observations have been made for the enantiomers of the long-acting β_2 -agonist formoterol, with the (R;R)-enantiomer being almost 1000-fold more potent than the (S;S)-enantiomer.¹⁰ The very high potency ratio for the enantiomers of formoterol has recently been confirmed on isolated human bronchus.¹⁷ It was further shown that in guinea pigs (R;R)-formoterol increased the ciliary beat frequency in the tracheal mucosa, a well-known effect of this class of drugs. (S;S)-Formoterol had virtually no effect in this preparation.¹⁸ The stereoselectivity of salmeterol, the other long-acting β_2 -agonist in clinical use, appears to be less marked, with an eudismic ratio around 50.¹⁹

For the partial β_2 -agonist clenbuterol, its agonistic and antagonistic activity at β -adrenoceptors was estimated on guinea pig trachea.²⁰ Again it was found that the (R)-enantiomer was about 1000 times more potent than the (S)-enantiomer as an agonist. The potency ratio for the antagonistic effect, inhibition of the relaxation by isoprenaline, was only about 100, suggesting a lower efficacy for (S)-clenbuterol compared with (R)-clenbuterol. That the distomer of a β -adrenoceptor agonist may have not only a lower potency but also a lower efficacy than the eutomer has been observed for TA-2005 as well.²¹ This compound is structurally related to formoterol and was developed as the pure (R;R)-enantiomer. Additional support for differences in efficacy between enantiomers of β -agonists stems from observations on human prostate PC3 cells.²² In this study the maximum cAMP production induced by salbutamol was 2 to 3 times higher for the (R)-enantiomer than for the (S)-enantiomer.

Extrapulmonary effects

Stimulation of β_2 -adrenoceptors in the skeletal muscle causes depression of subtetanic contractions of slow-

twitch fibers. This effect is associated with the muscle tremor, which appears as an unwanted side effect during treatment with β_2 -agonists.²³ For terbutaline,¹⁶ formoterol,¹⁰ clenbuterol,²⁰ and salbutamol,²⁴ it has been shown that this β_2 -adrenoceptor-mediated effect resides in the (R)-enantiomer.

The (R)-enantiomer of terbutaline, but not the (S)-enantiomer, inhibits microvascular leakage, as observed in a hamster cheek pouch preparation.²⁵ The stereoselectivity of this anti-inflammatory potential of terbutaline was confirmed in a study on canine forelimb, showing that (R)-terbutaline, but not (S)-terbutaline, inhibited histamine-induced increase in lymph flow and protein concentration.²⁶

In isolated guinea pig atria (β_1 - and β_2 -adrenoceptors) both enantiomers of salbutamol showed a positive chronotropic effect, with the (R)-enantiomer being about 200 times more potent than the (S)-enantiomer, an eudismic ratio similar to that observed on tracheal smooth muscle.²⁷ In the electrically driven papillary muscle (mainly β_1 -adrenoceptors) from guinea pig heart, (R)-terbutaline showed a positive inotropic effect, whereas the effect of (S)-terbutaline was negligible.¹⁶ For formoterol, both the chronotropic and inotropic effects were evaluated on preparations of the guinea pig heart in vitro. Only (R;R)-formoterol showed agonistic activity in relevant concentrations.¹⁰ Thus there is a comparable stereoselectivity for both β_1 - and β_2 -adrenoceptors. Clenbuterol showed no visible agonistic activity on the papillary muscle but exerted a β -antagonistic activity against isoprenaline.²⁰ In this action the (R)-enantiomer was again about 100 times more potent than the (S)-enantiomer.

In all of the studies on β -adrenoceptor agonists referred to above, covering a wide range of organs and functions, the effects displayed by the (S)-enantiomers were weak and related to interaction with β -adrenoceptors. No other effects were observed in relevant concentrations. The following is a review of investigations aimed at finding adverse effects of (S)-enantiomers unrelated to interaction with β -adrenoceptors.

ANIMAL STUDIES IN VIVO RELATING TO AIRWAY HYPERREACTIVITY

The first indication that the (S)-enantiomer of a racemic β -agonist might cause airway hyperreactivity originates from experiments on guinea pigs published 10 years ago.²⁸ This study showed that after infusion of either (R)- or (S)-isoprenaline for 1 hour and after the relaxing effect of (R)-isoprenaline had worn off, the airway reactivity to histamine increased to a comparable extent for both enantiomers.

An extended study carried out by the same research group further demonstrated that infusion of the racemate or either enantiomer of isoprenaline caused hyperreactivity to the peptide bombesin as well.²⁹ The hyperreactivity induced by *rac*-isoprenaline was shown to be dose dependent. Unfortunately, the separate enantiomers were given only at the highest dose, 100 $\mu\text{g} \times \text{kg}^{-1}$. At this dose level, (R)- and (S)-isoprenaline appeared to be equally active in

inducing hyperreactivity when calculated as changes in lung resistance. However, when the calculation was based on changes in dynamic lung compliance, a significant hyperreactivity was observed only after administration of (R)-isoprenaline. The induction of hyperreactivity induced by *rac*-isoprenaline was prevented by prior bilateral vagotomy, but the muscarinic antagonist atropine had no effect.

In a subsequent study by an independent research group, it was confirmed that after infusion of racemic or (R)-isoprenaline at a dose about half that used in the previously mentioned study, guinea pigs became hyperreactive to histamine.³⁰ However, these authors were not able to demonstrate hyperreactivity after administration of (S)-isoprenaline or an essential dependence on an intact vagus nerve for the hyperreactivity to develop. Taken together, these results suggest that the airway hyperreactivity induced by *rac*-isoprenaline is due to the action of the (R)-enantiomer and that the (S)-enantiomer may contribute, at least with higher doses.

Airway hyperreactivity in guinea pigs has been demonstrated also after intravenous infusion of (S)-salbutamol.³¹ In this study it was further shown that intratracheal instillation of 10 µg of (S)-salbutamol, (S)-terbutaline, or (S)-isoprenaline significantly increased airway reactivity to histamine, but only in animals sensitized to ovalbumin. It is disappointing that proper control experiments with the (R)-enantiomers were not performed. This should have been possible, at least for (R)-isoprenaline, with its transient bronchodilating effect.

In an attempt to further explore the role of (S)-enantiomers of β_2 -agonists, guinea pigs, normal or sensitized with ovalbumin, were exposed repeatedly to an aerosol of (S)-salbutamol for 4 days.³² The dose given was 20 times higher than the dose of *rac*-salbutamol required to cause bronchodilation. On the fifth day, the animals were challenged with histamine or allergen. There was no significant change in reactivity. This study, which has not yet been published in full, also suffers from the lack of proper control groups with the (R)-enantiomer, and we do not even know whether *rac*-salbutamol caused airway hyperreactivity during the prevailing experimental conditions.

ANIMAL STUDIES IN VITRO RELATING TO AIRWAY HYPERREACTIVITY

Reactivity to mediators

After preincubation with (S)-salbutamol (100 µmol/L for 90 minutes), increased reactivity to carbachol, but not to histamine or ovalbumin, was observed in smooth muscle preparations of trachea from guinea pigs sensitized to ovalbumin.³³ Parallel experiments with racemic or (R)-salbutamol showed no hyperreactivity but rather a residual inhibitory effect, probably caused by incomplete washing of the muscle strips. Under similar experimental conditions in human isolated bronchus, an increased response to histamine and leukotriene C₄, but not to methacholine, was observed after preincubation with 100 µmol/L (S)-salbutamol.³⁴ In contrast, the contractile response to anti-human IgE was attenuated by (S)-salbutamol.

Common to all these experiments is that very high concentrations have been used. This implies that traces of the (R)-enantiomer in the (S)-enantiomer may have contributed to the outcome of the experiments through, for example, a rebound effect. Control experiments with matching concentrations of the (R)-enantiomer and a washout time sufficient to eliminate the relaxing capacity are warranted. In this laboratory we were unable to find enhanced reactivity to carbachol in guinea pig trachea after exposure to (S;S)-formoterol or (S)-terbutaline. Nor did we find significant changes in reactivity to methacholine in a more integrated test system, the isolated perfused and ventilated guinea pig lung, after exposure to the diastomers of these two β_2 -agonists (unpublished data).

Reactivity to nerve stimulation

Hyperreactivity may also appear as an increased response to nerve stimulation. In guinea pig trachea and main bronchi, there are both cholinergic and noncholinergic excitatory nerves, which are activated by electrical field stimulation. (R;R)-Formoterol inhibited both cholinergic and noncholinergic contractile responses to nerve stimulation in a concentration-dependent manner.³⁵ (S;S)-Formoterol had qualitatively the same effect but was about 1000 times less potent. There was no indication that (S;S)-formoterol increased the response to nerve stimulation. Very similar results were obtained with the enantiomers of terbutaline.³⁵

The release of acetylcholine from cholinergic nerves, induced by electrical field stimulation, was studied on smooth muscle strips from equine trachea.³⁶ Racemic and (R)-salbutamol, as well as racemic and (R;R)-formoterol, caused a concentration-dependent increase in the release of acetylcholine. (S)-Salbutamol and (S;S)-formoterol had no effect. When the muscarinic autoreceptors (presynaptic M₂) were blocked by atropine, the release of acetylcholine was amplified, and the enhancing effect became significant also for (S;S)-formoterol. However, (S;S)-formoterol was at least 1000 times less potent than (R;R)-formoterol, as would be expected from a β_2 -adrenoceptor-mediated effect of this compound.¹⁰ Accordingly, the augmentation of acetylcholine release produced by both enantiomers of formoterol was inhibited by the β_2 -selective adrenoceptor antagonist ICI 118,551.³⁶ Very recently, it was reported that in airway tissue from horses with chronic obstructive pulmonary disease (COPD), (S;S)-formoterol augmented the release of acetylcholine, even in the absence of atropine, suggesting that in equine COPD, prejunctional M₂-receptors are dysfunctional.³⁷ In this communication, published in abstract form, no mention was made that (R;R)-formoterol may be 1000-fold more potent than (S;S)-formoterol as an enhancer of acetylcholine release in horses with COPD.

Effects on intracellular free calcium and interaction with muscarinic receptors

Another approach to study the effects of the enantiomers of β -adrenoceptor agonists in airway smooth muscle has been to measure changes in the intracellular

concentration of Ca^{2+} . Thus it was found that in isolated smooth muscle cells from bovine trachea, (S)-salbutamol, but not (R)-salbutamol, enhanced the increase in $[\text{Ca}^{2+}]_i$ induced by carbachol.³⁸ Using the same technique with fura-2-loaded cells, these authors further showed that the (S)-enantiomer and the racemate of salbutamol increased basal $[\text{Ca}^{2+}]_i$ in a concentration-dependent manner in the nanomolar range.³⁹ The (R)-enantiomer of salbutamol caused the intracellular Ca^{2+} concentration to decrease. Moreover, the increase in $[\text{Ca}^{2+}]_i$ caused by (S)-salbutamol (accompanied by an increase in inositol-1,4,5-triphosphate) was converted to a decrease by the muscarinic receptor antagonist atropine, suggesting a cholinergic component. In the presence of both atropine and the β_2 -selective adrenoceptor antagonist ICI 118,551, (S)-salbutamol had no effect on basal $[\text{Ca}^{2+}]_i$.

As further proof for the involvement of muscarinic receptors, (S)-salbutamol was found, in a binding assay, to compete with receptors for [^3H]-quinuclidinyl benzilate in the micromolar range.³⁹ This is in line with a very recent study demonstrating interaction with muscarinic receptors for a number of β -adrenoceptor agonists, including the racemates of salbutamol and formoterol.⁴⁰ However, whereas the interaction of (S)-salbutamol with muscarinic receptors in airway smooth muscle cells appeared to be agonistic in nature,³⁹ the interaction of formoterol at those receptors was mostly antagonistic.⁴⁰ This points to significant differences in efficacy at muscarinic receptors between various β_2 -agonists. In both of the studies referred to, the unspecific ligand [^3H]-quinuclidinyl benzilate has been used, leaving open the question on subtype classification of the muscarinic receptors involved. For (R;R)-formoterol, the affinity for muscarinic receptors was 1000-fold lower than the affinity of this compound for β_2 -adrenoceptors.⁴⁰ Moreover, the high degree of stereoselectivity of formoterol for binding to β_2 -adrenoceptors (eudismic ratio about 400) was largely lost for binding to muscarinic receptors (eudismic ratio of approximately 5).

The functional significance of the interaction of β -agonists with muscarinic acetylcholine receptors and effects on $[\text{Ca}^{2+}]_i$ is not obvious. Data from experiments comparing the effects of the enantiomers of formoterol and terbutaline on cholinergic and noncholinergic nerve responses in electrically stimulated strip preparations from guinea pig trachea do not indicate an interaction with muscarinic receptors.³⁵ Previously, it has been shown in strips from bovine tracheal smooth muscle and with aequorin as Ca^{2+} indicator that isoprenaline increases $[\text{Ca}^{2+}]_i$.⁴¹ This effect was blocked by the β -adrenoceptor antagonist propranolol. It was not stated whether racemic or (R)-isoprenaline was used. However, the Ca^{2+} -mobilizing effect of isoprenaline was mimicked by the direct activator of adenylate cyclase, forskolin. In the presence of carbachol, isoprenaline caused a fall in $[\text{Ca}^{2+}]_i$. The authors concluded that there is no simple correlation between $[\text{Ca}^{2+}]_i$ in smooth muscle cells and the tone exhibited by muscle.

Effects on eosinophils

There are numerous reports emerging on the action of β -adrenoceptor agonists on inflammatory cells. In one of them,⁴² the effects of the enantiomers of salbutamol on the stimulated secretion of eosinophil peroxidase was studied on human eosinophils. Racemic and (R)-salbutamol at 1 $\mu\text{mol/L}$ inhibited the secretion of eosinophil peroxidase induced by formyl-met-leu-phe/cytochalasin B in eosinophils from normal and allergic subjects. There was no statistically significant effect of (S)-salbutamol, and the authors concluded that their data did not suggest that selective use of an (R)-enantiomer of salbutamol will be of selective benefit in preventing eosinophil degranulation for patients receiving β -adrenoceptor therapy.

In human eosinophils formyl-met-leu-phe also induces generation of superoxide anions. Isoprenaline and salbutamol (probably the racemates) caused a concentration-dependent inhibition of this process in acute experiments.⁴³ If, however, the cells were exposed to the β -agonist for 24 hours before the challenge, with or without dexamethasone, the inhibitory effect on the oxidative burst was converted to an increase.⁴⁴ In this latter study (R)- and (S)-isoprenaline were studied separately. It was found that the reversal of the inhibitory effect of isoprenaline on respiratory burst in human eosinophils was related to β -adrenoceptors and was mediated by the (R)-enantiomer. (S)-Isoprenaline was devoid of effect. An inhibitory effect of *rac*-salbutamol, as well as salmeterol, on superoxide production in human eosinophils was observed also when the eosinophils were stimulated with IL-5.⁴⁵ The inhibitory effect was associated with (R)-salbutamol, whereas (S)-salbutamol enhanced superoxide production on prolonged exposure in the same manner as (R)-isoprenaline in the experiment quoted above.⁴⁴ When all these observations in vitro on human eosinophils are weighed together, the alarm signal for a specific proinflammatory effect of the (S)-enantiomer is not felt strongly.

CLINICAL STUDIES

The first study with (S)-isoprenaline in human subjects to address the hyperreactivity hypothesis involved 10 patients with asthma.⁴⁶ FEV_1 and PC_{20} (histamine) were measured before and then repeatedly up to 7 hours after inhalation of 10 mg of (S)-isoprenaline. In 7 of the patients, changes of FEV_1 and PC_{20} after inhalation of (S)-isoprenaline were not distinguishable from placebo. In 2 patients FEV_1 dropped below baseline after an initial rise induced by (S)-isoprenaline. Only in one patient was a decrease in PC_{20} after inhalation of (S)-isoprenaline noted. No control experiment with pure (R)-isoprenaline was made, although the (S)-isoprenaline used in this study contained 3% of this active enantiomer. Thus the changes observed may alternatively be interpreted as a rebound bronchoconstriction caused by the (R)-enantiomer.

In a double-blind, placebo-controlled, parallel-group study, 40 patients with mild asthma were given nebulized

salbutamol, 200 µg of the racemate, 100 µg of either of the enantiomers, or the vehicle.¹¹ The sensitivity to methacholine was measured before and 20 or 180 minutes after the exposure to salbutamol. Twenty minutes after inhalation, both the racemate and the (R)-enantiomer inhibited the response to methacholine by about 2 doubling doses. As observed after 180 minutes, the protective effect of the racemate appeared to disappear more rapidly than that of (R)-salbutamol. The (S)-enantiomer of salbutamol caused, if anything, a slight increase in the response to methacholine. This single dose study appears to have been published in duplicate elsewhere together with more extensive comments.⁴⁷ Increased responsiveness to methacholine after exposure to (S)-salbutamol was observed also in normal subjects, although it is unclear from the meeting report whether this occurred after relevant doses.⁴⁸

More recently, a double-blind cross-over study was performed in 17 subjects with mild-to-moderate asthma.⁴⁹ Single nebulized doses of *rac*-salbutamol (200 µg), (R)-salbutamol (100 µg), (S)-salbutamol (100 µg), or placebo was followed by challenge testing. Racemic and (R)-salbutamol protected equally well against both direct (methacholine) and indirect (adenosine monophosphate) challenge. There was no effect of (S)-salbutamol compared with placebo.

Higher doses of salbutamol were used in a double-blind, randomized, 4-way crossover study in 12 asthmatic subjects.⁵⁰ Both FEV₁ and methacholine PC₂₀ were measured before and again 20 and 180 minutes after a single dose of nebulized *rac*-salbutamol 2.5 mg, (R)- or (S)-salbutamol 1.25 mg, and placebo. In addition, cardiovascular parameters were measured, and assessment of "restlessness" was made. (R)-Salbutamol and the racemate protected against methacholine challenge and increased FEV₁ to a comparable extent. (S)-Salbutamol had no significant effect on the respiratory parameters apart from a slight protection against methacholine at 20 minutes. Restlessness and increased pulse rate were seen 20 minutes after racemic and (R)-salbutamol, but not (S)-salbutamol. No other adverse events were observed.

A further evaluation of the extrapulmonary effects of inhaled salbutamol was made in 12 healthy volunteers.⁵¹ This study also included kinetic data. Cumulative doubling doses of nebulized (R)- and (S)-salbutamol (from 200 to 3200 µg) or the racemate (from 400 to 6400 µg) were given. Racemic and (R)-salbutamol produced a dose-related increase in muscle tremor and a decrease in plasma potassium, two well-known β₂-adrenoceptor-mediated effects. (S)-Salbutamol had no detectable effect on these parameters. This is in keeping with observations on skeletal muscle contractions in vitro, which show that for salbutamol,²⁴ as well as for terbutaline, formoterol, and clenbuterol,^{16,10,20} the (R)-enantiomer is responsible for the changes in contraction pattern. Racemic and (R)-salbutamol increased heart rate, whereas (S)-salbutamol had no effect.⁵¹ The plasma levels of (S)-salbutamol were consistently higher than those of (R)-salbutamol, an observation that emphasizes the lack of effect of (S)-salbutamol.

There is little information on the effect of repeated

exposure to (S)-salbutamol.⁴⁸ There is, however, a recent double-blind, placebo-controlled, parallel-group multicenter clinical trial comparing racemic with (R)-salbutamol (levalbuterol) in 362 patients with asthma.⁵² Nebulized salbutamol (0.63 or 1.25 mg of the [R]-enantiomer or 1.25 or 2.5 mg of the racemate) was given 3 times daily for 4 weeks. Racemic salbutamol was used as rescue medication, which may have been a confounding factor. The greatest increase in FEV₁ was seen after 1.25 mg of (R)-salbutamol. After 4 weeks, 1.25 mg *rac*-salbutamol was the least effective treatment. The effect of 0.63 mg (R)-salbutamol was comparable to the effect of 2.5 mg of the racemate, suggesting a potency ratio of 4, rather than the expected ratio of 2. With these doses, the systemic side effects were less marked for (R)-salbutamol than for the racemate, implying an improved therapeutic ratio.

However, there was no apparent difference in bronchodilating effect between the 0.63-mg dose of (R)-salbutamol and 1.25 mg of *rac*-salbutamol at the start of the study, and the single dose study referred to above⁵⁰ suggests dose equivalence for 1.25 mg of (R)-salbutamol and 2.5 mg of the racemate. This indicates that the doses chosen may have been at the upper part of the dose-response curve, which will jeopardize a correct potency comparison. At week 4, the predose FEV₁ value improved for patients receiving (R)-salbutamol or placebo, but not *rac*-salbutamol.⁵² The modest differences in lung function observed between the active treatment arms in this 4-week study do not support the view^{7,8} that the (S)-enantiomer of a racemic β₂-agonist seriously compromises the therapeutic effect of the (R)-enantiomer. The results do, however, indicate the need for further clinical trials, as has been pointed out in a recent editorial.⁵³

Two structurally quite new β₂-agonists, picumeterol⁵⁴ and TA-2005,⁵⁵ have been developed as pure enantiomers and have been subject to clinical trials. Picumeterol (the pure [R]-enantiomer) was compared with its racemate in a small study on atopic subjects with asthma.⁵⁶ The bronchodilator potencies of picumeterol and its racemate were similar, as was the occurrence of adverse effects. The development of picumeterol appears to have been discontinued because of low efficacy and a short duration of action. TA-2005 (the pure [R;R]-enantiomer), although very potent and long acting, showed an effect/side-effect pattern similar to that known for established, racemic β₂-agonists in an early clinical study.⁵⁷ Since then, full clinical reports on TA-2005 do not seem to have been published. Apparently, from a clinical point of view, a pure enantiomer per se is not a sufficient advantage compared with established racemic β-adrenoceptor agonists.

PHARMACOKINETICS

Although the therapeutic effect of bronchodilating β₂-agonists resides in the (R)-enantiomer, the majority of pharmacokinetic studies have used achiral methods of analysis. This means that differences in absorption, metabolism, and excretion of the individual enantiomers may lead to erroneous conclusions about plasma concen-

tration-response relations.⁵⁸ The situation is even more complicated because the stereoselectivity of the metabolism of β_2 -agonists is not uniform. This topic has been extensively covered in a recent review.⁵⁹ Therefore only a few examples will be given to illustrate the confusion.

After intravenous, but particularly after oral, administration of *rac*-salbutamol in human subjects, the (R)-enantiomer is more rapidly eliminated than the (S)-enantiomer, leading to plasma concentrations of the distomer up to 3 times higher than those of the eutomer.^{60,61} This difference in kinetics is due to a preferential sulphate conjugation of (R)-salbutamol, primarily in the liver and intestines.⁶² For terbutaline, the condition is the opposite, with a preferential sulphation of the (S)-enantiomer.⁶³ This explains the greater first-pass metabolism of the (S)-enantiomer observed for this compound, leading to plasma concentrations of the distomer that are only about half those of the eutomer after repeated oral dosing.^{64,65} These data show that accumulation of the distomer is not a general problem for racemic β_2 -agonists and that terbutaline has more favorable pharmacokinetic properties than salbutamol in this respect.

CONCLUDING REMARKS

For chiral drugs used as racemates, it is imperative to explore the pharmacology of the individual enantiomers and their interaction. Moreover, pure enantiomers should be the goal in the development of new chiral drugs. However, when a proper reevaluation of an established racemic drug has shown that the pure active enantiomer offers no benefit to the patient compared with the racemate, there is little reason to remove the less active enantiomer from the formulation.⁶⁶

The warning against the use of β_2 -adrenoceptor agonists as racemates was primarily based on some *in vivo* observations on guinea pigs, showing hyperreactivity after exposure to (S)-enantiomers of isoprenaline and salbutamol.⁷ It is remarkable that today, 10 years after the first report on the issue,²⁸ other research groups have not been able to confirm the original observations, although attempts have been made.^{30,32} Among all the diverse observations made *in vitro*, the finding that (S)-salbutamol increases the intracellular free Ca^{2+} in dissociated cells from airway smooth muscle, seemingly by means of a muscarinic receptor,³⁹ is the most challenging one. Whether this property is unique for salbutamol or whether it may be extrapolated to other members of this class of drugs remains to be elucidated. In any case, there is little evidence at present that (S)-enantiomers of β_2 -agonists should elicit a direct contractile power on intact airway smooth muscle in relevant concentrations.

The clinical studies on enantiomers of β_2 -agonists are focused on salbutamol. They show unequivocally that the pulmonary, as well as the unwanted extrapulmonary, effects are mediated by the (R)-enantiomer, in all probability through activation of β -adrenoceptors. The evidence presented so far that the (S)-enantiomer should exert a clinically significant adverse effect is meager.^{53,67} There are still open questions about the long-term use of

the pure (R)-enantiomer compared with the racemate of bronchodilating β_2 -adrenoceptor agonists. These questions call for thorough clinical investigation. However, regular use of the long-acting β_2 -adrenoceptor agonists *rac*-formoterol for 1 year⁶⁸ or *rac*-salmeterol for 24 weeks⁶⁹ in asthmatic patients taking inhaled glucocorticosteroids did not increase the number and severity of exacerbations but rather increased asthma control compared with monotherapy with the steroid.

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