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Steric Aspects of Agonism and Antagonism at β-Adrenoceptors: Synthesis of and Pharmacological Experiments With the Enantiomers of Formoterol and Their Diastereomers

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ABSTRACT The enantiomers of formoterol (R;R and S;S) and their diastereomers (R;S and S;R) were synthesized and purified using a new procedure which required the preparation of the (R;R)- and (S;S)-forms of *N*-(1-phenylethyl)-*N*-(1-(*p*-methoxyphenyl)-2-propyl)-amine as important intermediates. The enantiomeric purity obtained was greater than 99.3%, usually >99.7%. The four stereoisomers were examined with respect to their ability to interact in vitro with β-adrenoceptors in tissues isolated from guinea pig. The effects measured were (1) relaxation of the tracheal smooth muscle (mostly β_2), (2) depression of subtetanic contractions of the soleus muscle (β_2), and (3) increase in the force of the papillary muscle of the left ventricle of the heart (β_1). All enantiomers caused a concentration-dependent and complete relaxation of the tracheal smooth muscle which was inhibited by propranolol. The order of potency was (R;R) > > (R;S) = (S;R) > (S;S). There was a 1,000-fold difference in potency between the most and the least potent isomer. The presence of the (S;S)-isomer did not affect the activity of the (R;R)-isomer on the tracheal smooth muscle. Also on the skeletal and cardiac muscles (R;R)-formoterol was more potent than its (R;S)-isomer. The selectivity for β_2-adrenoceptors appeared to be slightly higher for the (R;R)-isomer than for the (R;S)-isomer. The potency of the (S;R)- and (S;S)-isomers on the papillary muscle was too low to be determined accurately. The present study shows that determination of enantiomeric ratios and conclusions regarding structure–effect relationships are critically dependent on a very high degree of stereochemical purity.

KEY WORDS: chiral separation, enantiomeric purity, tracheal smooth muscle, skeletal muscle, cardiac muscle, guinea pig, in vitro

INTRODUCTION

Formoterol is a highly potent and β_2-selective adrenoceptor agonist[1] with a long effect duration when inhaled.[2] Like most other bronchodilating compounds of this class it is structurally related to isoprenaline. However, formoterol ((±)-(R*;R*)-(*N*-[2-hydroxy-5-[1-hydroxy-2-[[2-(*p*-methoxyphenyl)-2-propyl]amino]ethyl]phenyl] formamide) (**VII**) has two asymmetric carbon atoms in the molecule making four stereoisomers possible. In this paper the asymmetric carbons are assigned in parentheses in the following order: benzylic carbon, β-phenethylic carbon, and, where relevant, asymmetric carbon in the N-protecting group. Chiral centres, of which the relative but not the absolute configuration is known, are labelled arbitrarily by prefixes R*. The four isomers have been synthesized and briefly examined for relaxing activity on the guinea pig trachea.[3] Thus it was found that the isomer with (R)-configuration at both chiral centres was the most potent, the others being 3–14 times less potent. All subsequent studies on formoterol, clinical and preclinical, appear to have been performed with the fumarate of the enantiomeric mixture (R;R + S;S) with the laboratory code BD40A.[4] Although the generic name formoterol refers to this specific enantiomeric mixture, for simplicity the name will be used for all four enantiomers in the following report.

The reliability of enantiomeric potency ratios (stereospecific index) obtained in pharmacological studies are critically dependent on the purity of the respective stereoisomer. For example, traces of an active enantiomer in a virtually inactive one may cause an overestimation of its pharmacological potency and erroneous conclusions about structure–activity relationships. Although this matter has been appreciated for a long time[5] it is seldom discussed in pharmacological reports on stereoselectivity, perhaps due to difficulties in obtaining pure and well-defined stereoisomers as well as analytical limitations.[6] However, advances have been made in methods for both stereospecific drug synthesis and stereoselective drug analysis.[7] This is fundamental to studies on stereoselectivity in pharmacodynamics and pharmacokinetics.

In the present study we have made a reappraisal of the stereoselectivity reported for the four enantiomers of formoterol. We now describe a novel adaption of the method of Murase et al.[3,8] for the synthesis of (R;R)-, (R;S)-, (S;R)-, and (S;S)-formoterol. The modified procedure required the preparation of the (R;R)- and (S;S)-forms of *N*-(1-phenylethyl)-*N*-(1-(p-

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methoxyphenyl)-2-propyl)amine (**III**) as important intermediates. The starting amine I determines the stereochemistry of the reaction products as is shown in Scheme 1. With the new, highly purified enantiomers we reexamined the relaxing effect on the guinea pig tracheal smooth muscle. Furthermore, the β_1/β_2-selectivity has been evaluated using isolated preparations of cardiac and skeletal muscle.[9]

MATERIAL AND METHODS

Chemistry

The synthesis of the enantiomers of formoterol was performed according to Scheme 1.

Batch 1: In a first experiment the commercially available (+)-(R)-1-phenylethylamine (**I**) containing 2% of the corresponding (S)-enantiomer was used as starting material. A rather poor enantiomeric purity was obtained in spite of a semipreparative chromatographic work-up procedure.

Batch 2: In the next experiment the same starting material was used, but in this case the (R;R)-amine **III** was further resolved with (R;R)-tartaric acid. However, the enantiomeric purity was still too low to be acceptable.

Batch 3: The low enantiomeric purity obtained in earlier batches forced us to purify the (R)-amine **I** by crystallization using (S;S)-tartaric acid as the resolving agent, giving an enantiomeric purity of 99.9% of the amine **I**. The pure (R)-enantiomer **I** was then reductively alkylated with 4-methoxyphenyl-2-propanone (**II**) and hydrogen over Raney Nickel catalyst at 345 kPa to give the (R;R)-amine **III**. This amine was then treated with (R;R)-tartaric acid and further resolved by fractional crystallization from ethanol, giving a product where (S;S)-amine was less than 0.1% as detected on a chiral α_1-acid glycoprotein (AGP) HPLC-system.

The same procedures as in batch 1–3 were used for the corresponding (S)-amine **I** using (R;R)-tartaric acid as the resolving agent giving a purity of 99.9%. The (S;S)-amine **III**, obtained by the same sequence as the (R;R)-amine, gave after crystallization of its (S;S)-tartaric acid salt, an enantiomeric purity of 99.6%.

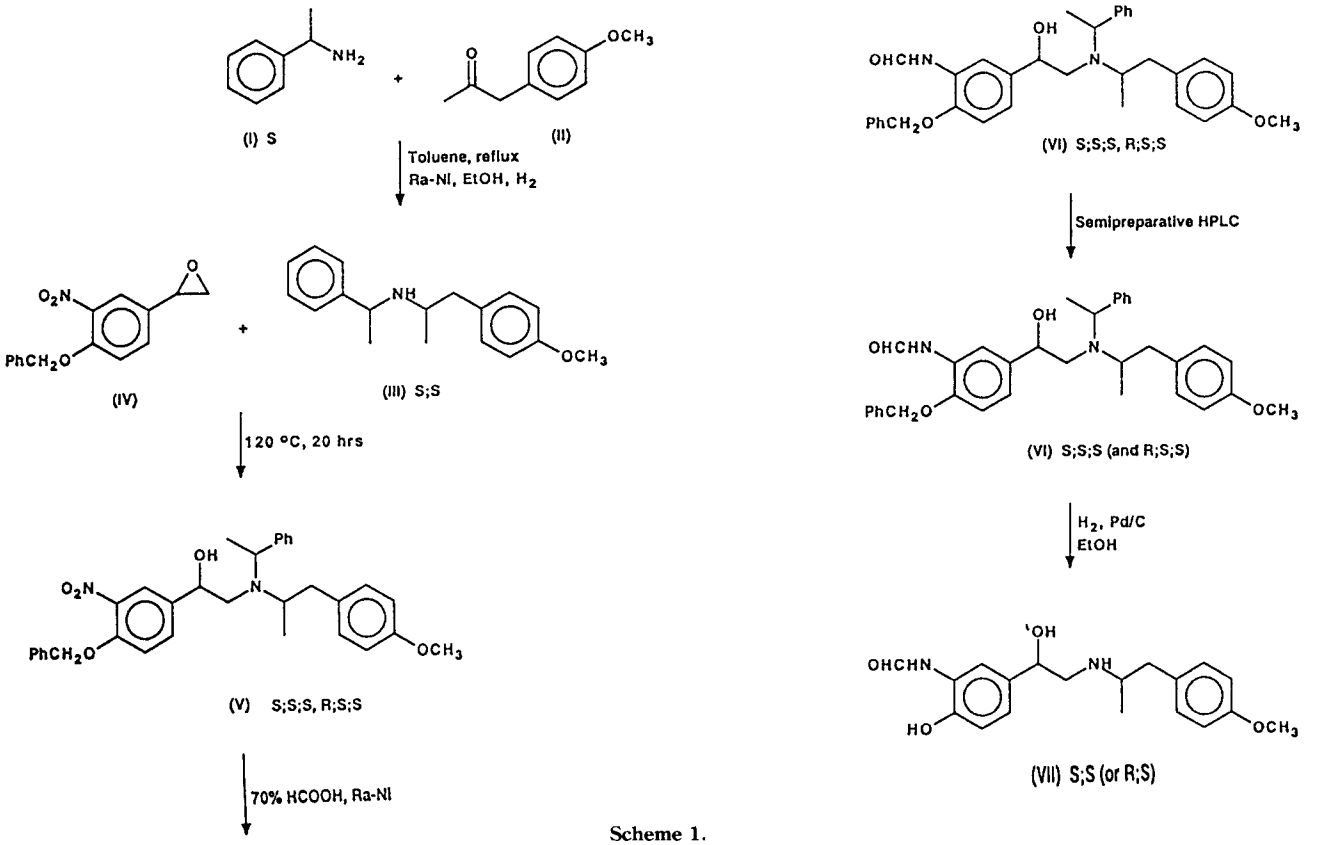
4-Benzyloxy-3-nitro-α-[[*N*-(1-phenylethyl)-*N*-(1-(*p*-methoxyphenyl)-2-propyl)amino]benzyl alcohol (**V**) was obtained by reacting 4-benzyloxy-3-nitrostyrenoxide (**IV**) with the amine (**III**) in the absence of solvent at 140°C for 20 h. The nitro group in (**V**) was reduced and formylated in aqueous formic acid with Raney Nickel[10] to give the crude formanilide (**VI**) which was purified on a silica chromatographic system.

The diastereomers of **VI** were then separated on a semipreparative chromatographic system using a C_{18} column.

Each isomer was then hydrogenated in the presence of 10% Pd/C in ethanol at room temperature and 345 kPa giving *N*-[2-hydroxy-5-[1-hydroxy-2-[[2-(*p*-methoxyphenyl)-2-propyl]amino]ethyl]phenyl]formamide (**VII**).

The same procedure was used for the synthesis of the remaining isomers, R;R and S;R, respectively.

The NMR spectra were recorded on a Varian VRX-300 spectrometer using tetramethylsilane (or d_6-DMSO) as an internal standard and the indicated solvent at ambient temperature.



Scheme 1.

Mass spectra were recorded on a Hewlett-Packard 5890/5970 GC/MS spectrometer. High-pressure semipreparative liquid chromatography was performed with a Gilson equipment using a Dynamax-60A, C_{18} column with a C_{18} precolumn. Column chromatography was done with Merck Kieselgel 60 (0.040-0.063 mm). The primary amine I was analyzed by reaction with the bifunctional reagent 1,5-difluoro-2,4-dinitrobenzene with a subsequent chromatographic step on a C_{18} column using acetonitrile (70%)/0.01 mol/liter phosphate buffer (30%), pH 7 (L. Hansson, to be published).

The amines **III** were analyzed on a reversed C_{18} system using acetonitrile (40%)/0.1 mol/liter triethylamine + 0.01 mol/liter phosphate (60%), pH 7 as eluent. The enantiomeric purity was analyzed on a chiral AGP-column using methanol (15%)/10 mmol/liter phosphate buffer (85%), pH 6.35 as eluent.

The final products were analyzed on a C_{18} column using acetonitrile (20%)/50 mmol/liter phosphate buffer (80%), pH 3 as eluent. The main peak from this reversed phase system was then further evaluated for enantiomeric purity on a chiral AGP column using methanol (5%)/10 mmol/liter phosphate buffer (95%), pH 6.75 as eluent.

Purification of (+)-(R)-1-phenylethylamine (**I**) and (−)-(S)-1-phenylethylamine (**I**)

Commercially available (+)-(R)-1-phenylethylamine I (0.1 mol, 12.1 g) and (S;S)-tartaric acid (0.1 mol, 15.0 g) were dissolved in hot water (50 ml). Cooling and standing overnight gave crystals. The crystallization procedure was repeated three times. Extracting the base between ethyl acetate and ammonia (1 mol/liter) gave 4.8 g of the amine **I** (yield 39%, enantiomeric purity >99.9%) after drying and evaporation of the solvent. The same procedure was used for purification of the (−)-(S)-1-phenylethylamine **I** with (R,R)-tartaric acid giving a yield of 4.1 g (34%, enantiomeric purity >99.9%).

N-(1-Phenylethyl)-*N*-(1-(*p*-methoxyphenyl)-2-propyl)amine (**III**)

A mixture of (−)-(S)-1-phenylethylamine **I** (30 mmol, 4.1 g) and *p*-methoxybenzyl methyl ketone **II** (30 mmol, 5.6 g) was refluxed in toluene (100 ml) until all water formed had been collected (5–6 h). After evaporation of the solvent the residue was dissolved in ethanol (100 ml) and Raney-Nickel (6 g) was added. The solution was hydrogenated at 345 kPa overnight. Removal of the catalyst by filtration and evaporation of the solvent gave the product **III**. To this (S;S)-amine was added (S;S)-tartaric acid (30 mmol, 4.2 g) in hot ethanol (50 ml). The crystals obtained were recrystallized once in ethanol (100 ml) giving a product with an enantiomeric purity of 99.6%; no (R;R)-amine detected. Extracting the base between ethyl acetate and ammonia (1 mol/liter) gave the pure (S;S)-amine **III** in a total yield of 4.5 g (56%). The (R;R)-amine was obtained from (+)-(R)-1-phenyl-ethylamine **I** in a yield of 3.3 g (31%) with a chemical purity of 97.7%; no (S;S)-amine was detected.

N-[2-Hydroxy-5-[1-hydroxy-2-[[2-[*p*-methoxyphenyl]-2-propyl]amino]ethyl]phenyl]formamide (**VII**)

The (S;S)-amine **III** (16.7 mmol, 4.5 g) and 4-benzyloxy-3-nitrostyrene oxide (**IV**) (18.7 mmol, 4.8 g) were heated under stirring in a nitrogen atmosphere at 140°C for 20 h. After cooling 70% formic acid (100 ml) and Raney-Nickel (9 g) was added to the sirup, during stirring for 1 h at 100°C. The catalyst was removed by filtration and washed with boiling water. The solution was extracted with ethyl acetate/ammonia (1 mol/liter), the organic phase washed with water, dried over sodium sulphate, and evaporated. Chromatography of the residue on a silica column using petroleum ether/ethyl acetate (3:2) was followed by a semipreparative chromatographic step on a Supercosil C_{18} column using a mixture of 50% acetonitrile and 50% ammonium acetate (0.1 mol/liter) in acetic acid (0.1 mol/liter). The first peak consisted of the (S;S;S)- and the second of the (R;S;S)-enantiomer. Each fraction was extracted between ethyl acetate/ammonia (1 mol/liter). The organic phase was washed with water, dried, and evaporated. The (S;S;S) fraction was dissolved in ethanol (50 ml), 0.2 g 10% Pd/C was added, and the mixture was hydrogenated for 5 h at 345 kPa. After filtration, evaporation of the solvent, a residue of **VII** (0.273 mmol, 94 mg) was obtained.

Fumaric acid (0.138 mmol, 16 mg) was added to the residue dissolved in methanol. Evaporation of the solvent gave the product (S;S) **VII** semifumarate (109 mg) characterized by ^{1}H-NMR (d_6-DMSO) δ (ppm) 1.00 (d, 3H, CHC$\underline{H}_3$), 4.62–4.70 (m, 1H, C$\underline{H}$OH), 3.73 (s, 3H, O$\underline{CH}_3$), 6.8–6.9 (m, 3H, aromatic), 7.00 (dd, 4$\underline{H}$, aromatic), 6.49 (s, 1$\underline{H}$, C$\underline{H}$=C$\underline{H}$; fumarate). MS of disilylated (S;S) **VII**: 473 (M^+–CH_3, 7%); 367 (M^+–C_8H_9O, 45%); 310 (M^+–$C_{11}H_{16}NO$, 17%); 178 ($C_{11}H_{16}NO$, 95%); 121 (C_8H_9O, 61%). The (R;S;S) fraction was treated in the same manner giving the product (R;S) **VII** semifumarate, which was characterized by ^{1}H-NMR (d_6-DMSO) δ (ppm) 1.01 (d, 3H, CHC$\underline{H}_3$), 3.76 (s, 3H, O$\underline{CH}_3$), 6.49 (s, 1H, C$\underline{H}$=C$\underline{H}$, fumarate) 6.8–6.9 (m, 3H, aromatic), 7.0 (dd, 4H, aromatic). MS of disilylated (R;S) **VII**: 473 (M^+–CH_3, 5%); 367 (M^+–C_8H_{90}, 48%); 310 (M^+–$C_{11}H_{16}NO$, 18%); 178 ($C_{11}H_{16}NO$, 95%); 121 (C_8H_{90}, 52%). The structural data for the (R;R) and (S;R) enantiomers were in accordance with the proposed structures. The enantiomeric purity obtained for the enantiomers in each batch is shown in Table 1.

Pharmacology

Male Dunkin–Hartley guinea pigs about 250 g kept under standard laboratory conditions were used in all experiments.

TABLE 1. Enantiomeric purity for the enantiomers of formoterol and their diastereomers

	Enantiomeric distribution (%)			
Enantiomer	(R;R)	(R;S)	(S;R)	(S;S)
Batch 1				
(R;R)	98.6	<0.2	0.1	1.2
(R;S)	0.9	97.2	1.6	0.3
(S;R)	<1.0	1.8	97.4	0.4
(S;S)	1.5	0.2	0.6	97.8
Batch 2				
(R;R)	98.0	<0.1	<0.5	1.5
(R;S)	2.9	94.1	2.3	0.8
Batch 3				
(R;R)	>99.8	<0.1	<0.1	<0.1
(R;S)	<0.1	99.7	<0.1	0.2
(S;R)	<0.2	<0.1	>99.7	<0.1
(S;S)	<0.1	0.5	<0.1	>99.3

The animals were stunned by a blow on the neck and exsanguinated. The heart, trachea, and soleus muscles were dissected out, transferred to dishes with oxygenated Krebs solution, and prepared as described below. Measurements of isometric contractions were made with the aid of a Grass model 7D Polygraph using FT03 force transducers. The contractions of the spontaneously beating right atrium triggered a tachygraph. Electrical pulses for stimulation of the papillary and soleus muscles were provided by a Grass S88 or S48 stimulator via an SIU5 isolation unit. All experiments were performed in water-jacketed organ baths (40 ml) containing Krebs solution of the following composition in mmol/liter: NaCl, 118; KCl, 4.7; $CaCl_2$,2.5; $MgSO_4$, 1.16; $NaHCO_3$, 25; KH_2PO_4, 1.18; D-glucose, 11.1. The solution was maintained at 37°C and oxygenated with a mixture of 5% CO_2 in O_2. Each preparation was used for only one experiment.

The *trachea* was freed from connective tissue and cut into sections of two cartilage rings. Cotton threads were fastened to both ends of the cartilage bridge, which was then cut open ventrally. The preparation, mounted at a basal tone of 5 mN, was allowed to stabilize for 1 h before the start of the experiment.[9] Tracheal tone was induced by carbachol, 0.1 μmol/liter, or histamine, 0.5 μmol/liter. Relaxant effects were calculated in per cent of the maximum relaxation by 1 mmol/liter theophylline added at the end of the cumulative addition of the test compound.

The *papillary muscle* from the left ventricle of the heart was dissected out and mounted on a holder at a basal tone of 5 mN. The muscle was paced with maximum pulses of 1 msec duration at a frequency of 3 Hz.[9] The positive inotropic effect was expressed in percent of the maximum increase produced by 10 μmol/liter isoprenaline added at the end of the cumulative addition of the test compound.

The *right atrium* of the heart was freed from ventricular tissue and mounted on a holder at a basal tone of 5 mN. The preparation was spontaneously beating with a basal frequency of about 3.5 Hz.[11] The positive chronotropic effect was expressed as a percentage of the maximum increase produced by 10 μmol/liter isoprenaline added at the end of the cumulative addition of the test compound. The positive inotropic effect was calculated as well.

The *soleus muscle* was prepared essentially as described previously.[12] Subtetanic contractions (S) were evoked by transmural field stimulation (maximum pulses 0.5 msec, about 12 Hz for 1.5 sec) every 20 sec. Each train of pulses was followed, with 10 sec delay, by a single pulse producing a twitch (T). The ratio S/T was used to calculate the degree of fusion of the subtetanic contractions.[13] The effects were expressed in percent of the maximum depression produced by 10 μmol/liter isoprenaline added at the end of the cumulative addition of test substance.

The *drugs used* and their sources were (−)-isoprenaline hydrochloride and carbamylcholine (carbachol, Sigma Chemical Co.), histamine chloride (Apoteksbolaget), (±)-propranolol hydrochloride (ICI), and theophylline (AB Draco). The stereoisomers of formoterol were prepared in our laboratories as described above. Solutions were made up in saline, when necessary with the aid of a few drops of glacial acetic acid. pH was adjusted to 7.4 with NaOH.

Statistical evaluation was made using Student's *t* test.

RESULTS

Enantiomeric Purity

The enantiomeric purity of the various batches are shown in Table 1. In batch 1 the commercial amine **I** has been used without further purification, in batch 2 the secondary amine **III** has been purified by fractional crystallization with tartaric acid and in batch no 3 a purification step has been used at each level. The pharmacological studies have run in parallel with efforts in the chemistry department to improve the stereochemical purity. Therefore some experiments have been performed using batches with less than optimal purity, but this will appear from the presentation of the results.

The small amount available of each isomer did not permit a precise measurement of the optical rotation, but a rough estimation has been made. Thus the (S;S)- and (S;R)-isomers were found to be dextrorotatory while the (R;R)- and (R;S)-isomers were levorotatory.

Effects on Tracheal Strips

All four stereoisomers of formoterol caused a concentration-dependent and complete relaxation of the tracheal smooth muscle, independently of whether tracheal tone was induced by carbachol, 0.1 μmol/liter, or histamine, 0.5 μmol/liter (Figs. 1 and 2). There were no apparent differences in the pD_2 values obtained under the two different experimental conditions (first batches compared, Table 2). Propranolol, 0.1 μmol/liter, moved the concentration–response curves, obtained in the presence of histamine, for all four isomers of formoterol two log units to the right ($P < 0.001$) with no change in maximum relaxation (Fig. 2).

There were only minor differences in the pD_2 values between the different batches of a specific stereoisomer with one exception: batch 3 of (S;S)-formoterol was 10 times less active than batch 1 ($P < 0.001$) which contained at least 10 times more of the highly potent (R;R)-isomer. The data in Table 1 also suggest that batch 3 of (S;R)-formoterol may be more potent than batch 1. Since seasonal variations in sensitivity of the

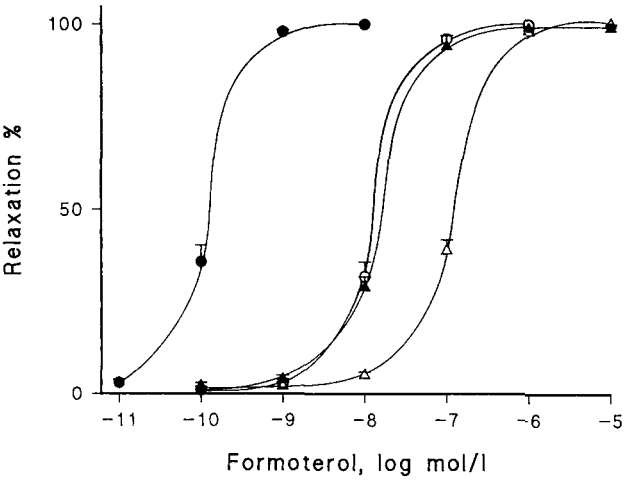


Fig. 1. Relaxation of the carbachol-contracted tracheal smooth muscle by the four stereoisomers of formoterol. The effects (means ± SE of 5–6 experiments) are expressed in percent of the maximum relaxation by theophylline. (●) (R;R) batch 3, (○) (R;S) batch 3, (▲) (S;R) batch 3, and (△) (S;S) batch 3.

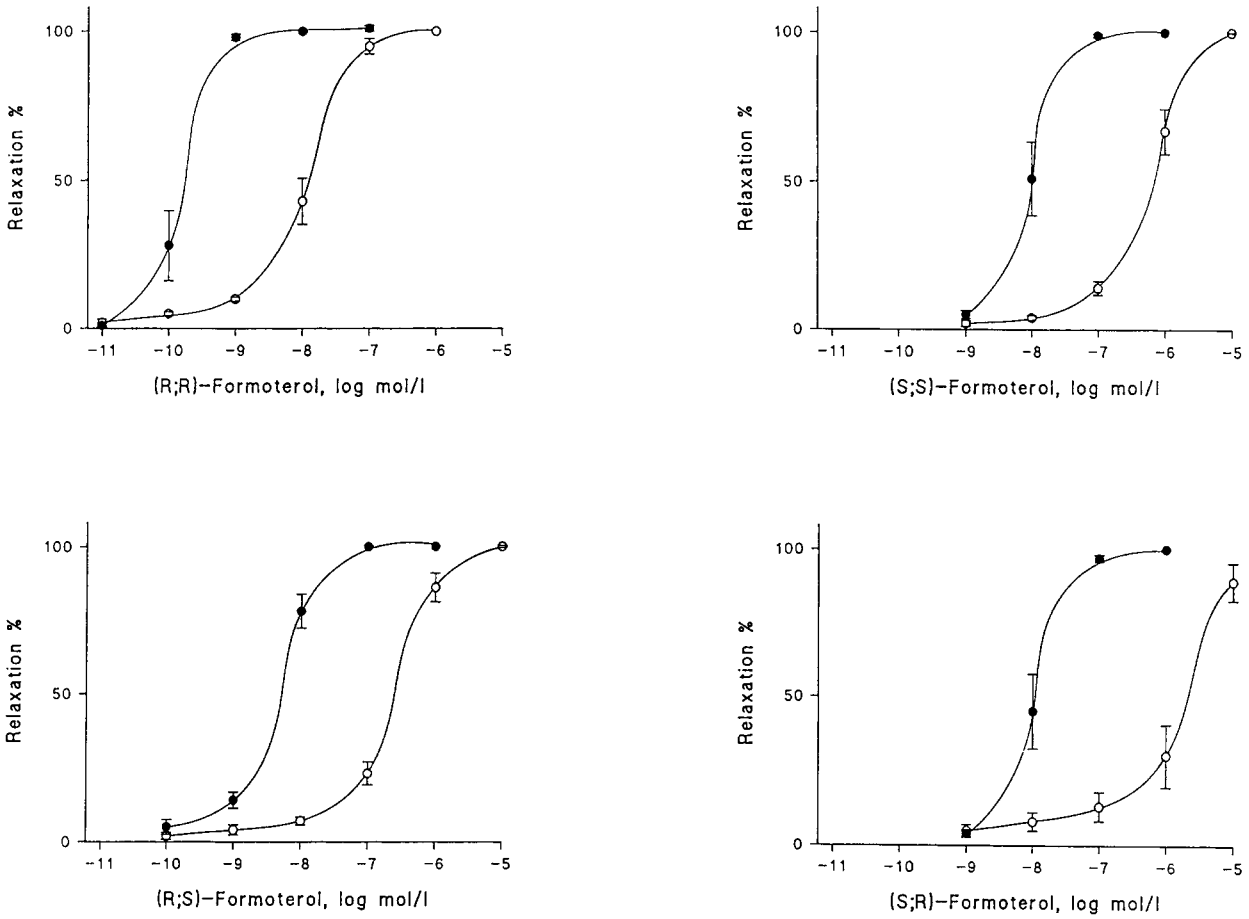


Fig. 2. Relaxation of the histamine-contracted tracheal smooth muscle by the four stereoisomers of formoterol and its inhibition by propranolol. Cumulative concentration–effect curves were obtained with (○) and without (●) 0.1 μmol/liter propranolol. The effects (mean ± SE of 5 experiments) are expressed in percent of the maximum relaxation by theophylline. Batch number 1 of each isomer was used.

tracheal smooth muscle cannot be excluded the experiments were repeated, matched in time. The pD_2 values for batch 1 and batch 3 of (S;R)-formoterol were then 7.61 ± 0.08 and 7.57 ± 0.09 (mean ± SE of 5–6 experiments), respectively.

If data from the most pure batches are considered the order of potency is (R;R) > > (R;S) = (S;R) > (S;S). There was an almost 1,000-fold difference in potency between the most and the least potent isomer ($P < 0.001$) while the greatest interval in the series is the 100-fold difference between the (R;R)- and the (R;S)-isomer ($P < 0.001$). Moreover, the 7-fold potency difference between the (S;R)- and the (S;S)-isomer was highly significant ($P < 0.001$).

In order to detect a possible interaction between the enantiomers (R;R) and (S;S), the relaxing capacity of (R;R)-formoterol on carbachol (0.1 μmol/liter) contracted tracheal strips was examined with and without 10 nmol/liter (S;S)-formoterol in the bathing medium. The pD_2 values obtained were 9.69 ± 0.08 and 9.76 ± 0.02 (mean ± SE of 5–6 experiments), respectively. There was no difference in maximum relaxation.

TABLE 2. Relaxation by the four stereoisomers of formoterol of the guinea-pig trachea[a]

	Batch[b]	(R;R)	(R;S)	(S;R)	(S;S)
Carbachol	1	9.63 ± 0.04 (6)	8.11 ± 0.15 (6)	7.30 ± 0.10 (9)	7.90 ± 0.06 (5)
	2	10.03 ± 0.02 (6)	8.19 ± 0.13 (5)	—	—
	3	9.89 ± 0.04 (6)	7.87 ± 0.06 (6)	7.83 ± 0.06 (5)	6.96 ± 0.02 (6)
Histamine	1	9.79 ± 0.12 (5)	8.36 ± 0.10 (5)	7.98 ± 0.12 (5)	8.06 ± 0.14 (5)
+ propranolol	1	7.87 ± 0.10 (5)	6.64 ± 0.12 (5)	5.68 ± 0.19 (5)	6.18 ± 0.09 (5)

[a]Contraction was induced either by 0.1 μmol/liter carbachol or 0.5 μmol/liter histamine with or without 0.1 μmol/liter propanolol. The data are the mean pD_2 ± SE with the number of experiments in parentheses.
[b]For specifications see Table 1.

Effects on Skeletal and Cardiac Muscle

Formoterol caused a concentration-dependent depression of the subtetanic contractions of the soleus muscle (Fig. 3). In this respect the (R;R)-isomer was about 15 times more potent than the (R;S)-isomer ($P < 0.001$; batch 1 and batch 3, respectively, compared) (Table 3). Isoprenaline, 10 μmol/liter, added at the end of the cumulative addition of the test compound did not depress the soleus muscle further. There was no statistically significant difference between the two batches of (R;S)-formoterol tested.

In the electrically driven papillary muscle the (R;R)- and (R;S)-isomers of formoterol increased the force of contraction. This effect was also concentration dependent and reached the same maximum as that achieved by 10 μmol/liter isoprenaline (Fig. 3), but the (R;R)-isomer was about 10 times more potent than the (R;S)-isomer ($P < 0.005$; batch 1 and batch 3, respectively, compared) (Table 3). There were no statistically significant differences between the various batches of either isomer. The (S;R)- and (S;S)-isomers increased the force of contraction from about 1 μmol/liter but this effect was at higher concentrations counteracted by a negative inotropic effect (data not shown).

TABLE 3. Depression of subtetanic contractions of the soleus muscle and increase in force of the electrically driven papillary muscle from guinea pig by two stereoisomers of formoterol[a]

Tissue	Batch[b]	(R;R)	(R;S)
Soleus muscle	1	9.26 ± 0.08 (8)	7.77 ± 0.28 (5)
	3		8.08 ± 0.10 (6)
Papillary muscle	1	6.81 ± 0.15 (5)	
	2	6.79 ± 0.09 (5)	6.10 ± 0.10 (5)
	3		5.78 ± 0.17 (6)

[a]The data are the mean $pD_2 \pm SE$ with the number of experiments in parentheses.
[b]For specifications see Table 1.

In the spontaneously beating right atrium (R;R)-formoterol caused a concentration-dependent increase in both rate and force reaching the same maximum as 10 μmol/liter isoprenaline (Fig. 4). The mean $pD_2 \pm SE$ for rate was 8.17 ± 0.17 ($n = 6$) and for force 7.14 ± 0.37 ($n = 5$). Thus there was a 10-fold difference in potency between the chronotropic and inotropic effects of (R;R)-formoterol ($P < 0.025$).

DISCUSSION

The present study confirms that of the four stereoisomers of formoterol the (R;R)-isomer is the most potent in relaxing the tracheal smooth muscle, followed by the (R;S)- and then closely by the (S;R)- and the (S;S)-isomers.[3] The relaxing capacity was the same whether tracheal tone was induced by carbachol or histamine, an indication of functional antagonism. Furthermore, propranolol shifted the concentration–response curves almost two log units to the right throughout which indicates that the relaxing effect of all four isomers is mediated via β-adrenoceptors.

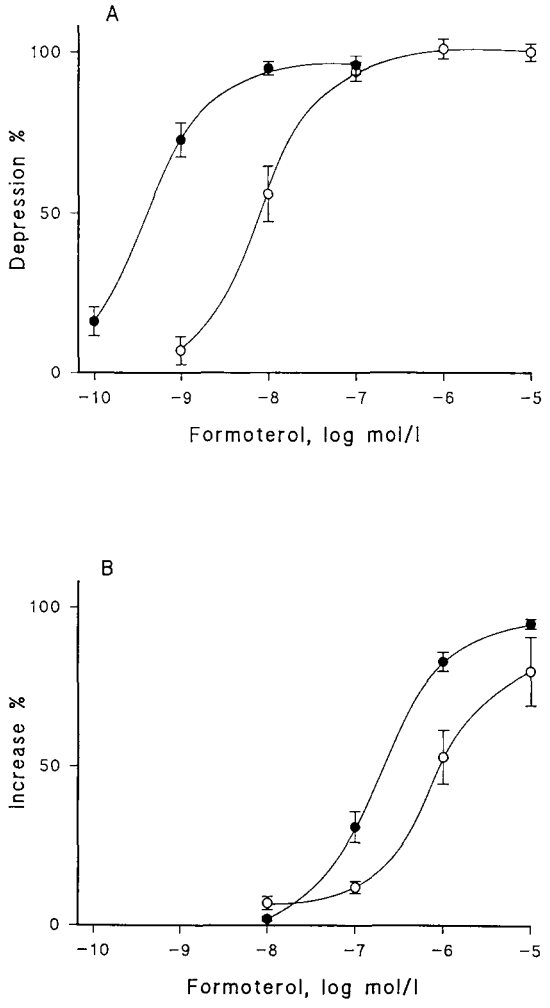


Fig. 3. Effects of (R;R)- and (R;S)-formoterol on skeletal and cardiac muscle contractions. (**A**) Depression of subtetanic contractions of the soleus muscle and (**B**) increase of the force of the electrically driven papillary muscle. The effects (mean ± SE of 5–8 experiments) are expressed in percent of the maximum effect of isoprenaline. (●) (R;R), batch 1; (○) (R;S), batch 3.

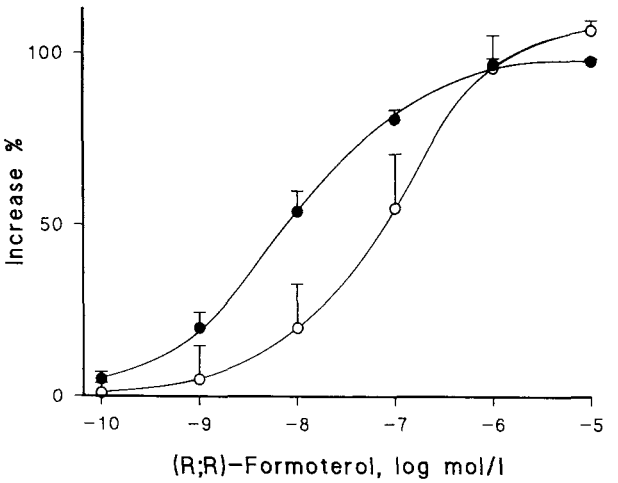


Fig. 4. Effect of (R;R)-formoterol on rate and force of the spontaneously beating right atrium of the heart. The effects (mean ± SE of 5–6 experiments) are expressed in percent of the maximum effect of isoprenaline. (●) Rate and (○) force. Batch 1 of (R;R)-formoterol was used.

We found an up to 40 times higher difference in potency between the (R;R)- and (R;S)-isomers on the tracheal smooth muscle than did Murase et al.[3] which may be due to a higher stereochemical purity of our compounds. Our first batches of the (R;S)- and (S;S)-isomers contained 1–2% of the highly potent (R;R)-isomer of formoterol while the upgraded batches contained $< 0.1\%$. This improvement in quality resulted at most in a slight increase in the estimated EC_{50} value for the (R;S)- but a 10-fold increase of the EC_{50} for the (S;S)-isomer. Evidently most of the activity of the first batch of the (S;S)-isomer was due to the traces of the active (R;R)-isomer. Thus our estimates of the EC_{50} values for the (R;R)- and (S;S)-isomers ended up with an almost 1,000-fold difference which should be compared with the fourfold difference reported by Murase et al.[3] Still it cannot be excluded that part of the activity displayed by (S;S)-formoterol in batch 3 is related to traces of (R;R)-formoterol. From the foregoing follows that a high degree of stereochemical purity is essential for reliable conclusions on structure–activity relationships, particularly when high enantiomeric potency ratios are at hand. This limitation should be born in mind in the following discussion.

Formoterol belongs to a wide range of β-adrenoceptor ligands which are derivatives of isoprenaline, substituted in the isopropyl moiety. Of these compounds the antagonist labetalol[14,15] and the agonist PTFMA, a *p*-trifluoromethylanilide congener of isoprenaline,[16,17] have been subject to a more detailed study on their stereoselectivity. For both these compounds it was found that the (R;R)-isomer was the most potent. When the configuration was changed in the isopropyl moiety as in the (R;S)-isomer there was a marked drop in potency similar to what we observed for formoterol.

Interestingly, for all three compounds there are only minor potency differences between the (R;S)-isomer and those with (S)-configuration at the carbon atom carrying the 2-hydroxyl group, i.e., the (S;R)- and (S;S)-isomers. This means that the favourable influence of the (R)-configuration at this site[18] is counteracted by an unfavourable configuration of the nitrogen substituent. In this context it is interesting to recall that the conversion of α-methyldopamine to α-methylnoradrenaline, another phenylethanolamine, by the enzyme dopamine β-hydroxylase is hampered when the α-methyl group has the "wrong" configuration.[19]

While relaxation of the guinea pig tracheal smooth muscle is mediated via β_2-adrenoceptors together with a small fraction of β_1-adrenoceptors,[20,21] the positive inotropic effect on the papillary muscle appears to be mediated almost exclusively via β_1-adrenoceptors.[9,22] Conversely, the positive chronotropic effect on the right atrium is mediated via β_1-adrenoceptors and a minor fraction of β_2-adrenoceptors.[11] The depression of subtetanic contractions of the guinea pig soleus muscle is a pure β_2-adrenoceptor mediated effect.[23,24]

Both (R;R)- and (R;S)-formoterol show a high degree of selectivity for β_2-adrenoceptors. A closer inspection of our data on the trachea (mainly β_2) and on the papillary muscle (β_1) might suggest that the selectivity quotient β_2/β_1 is greater for the (R;R)- than for the (R;S)-isomer. However, this difference becomes less obvious when the comparison is made on data obtained on the soleus muscle (β_2). The β_2-selectivity of the (S;R)- and (S;S)-isomers was difficult to evaluate due to unspecific effects of the high concentrations required for the papillary muscle. In any case, conformational changes in the nitrogen substituent of a β-adrenoceptor ligand appear to affect potency more than selectivity. This view is supported by data on labetalol[15] and PTFMA.[16] On the other hand, experiments with the stereoisomers of a series of phenylethanolaminotetralines show a more complex relation between potency changes and selectivity for β-adrenoceptor subtypes.[25]

On the spontaneously beating right atrium (R;R)-formoterol was 10 times more potent as a chronotropic than an inotropic agent. Similar observations have been made for racemic formoterol[1] and for another highly potent and selective β_2-adrenoceptor agonist, procaterol.[26] This phenomenon may be attributed to a significant population of β_2-adrenoceptors in the right atrium of the guinea pig heart contributing to the chronotropic response.[11]

The present data indicate that the pharmacodynamic activity of rac. formoterol is exerted by the (R;R)-enantiomer. Since the (S;S)-enantiomer is practically inactive there is from this point of view no reason for its removal from the racemate in pharmaceutical preparations according to the decision tree suggested by Testa and Trager.[27] Moreover, our study verifies the prediction by Barlow et al.[5] that there is a subtle relation between the biological activity and the degree of resolution of optical isomers.

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