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Steric Aspects of Formoterol and Terbutaline: Is There an Adverse Effect of the Distomer on Airway Smooth Muscle Function?

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ABSTRACT Experiments were made on isolated tissues from guinea-pig to test the hypothesis that the distomers of *rac*-β_2-adrenoceptor agonists induce airway hyperreactivity. Tracheal strip preparations were contracted with carbachol. Both *rac*- and (R;R)-formoterol (2 and 1 μmol/l, respectively) produced an immediate relaxation, followed by a slow recovery of tone. (S;S)-Formoterol (2 μmol/l) had no effect on smooth muscle tone. Similar results were obtained with the enantiomers of terbutaline. In other strip preparations of the trachea or the main bronchi, cholinergic or nonadrenergic/noncholinergic (NANC) excitatory responses were evoked by electrical field-stimulation. The eutomers, (R;R)-formoterol and (R)-terbutaline, inhibited concentration-dependently both cholinergic and NANC-induced contractions. The distomers, (S;S)-formoterol and (S)-terbutaline, showed qualitatively the same effects but were about 1,000 times less potent than the corresponding eutomer. In a third series of experiments, either enantiomer of formoterol was administered to an electrically stimulated vagus nerve-trachea tube preparation. The nerve-induced contractions were inhibited by both enantiomers, but (S;S)-formoterol was about 1,000 times less potent than (R;R)-formoterol. For both enantiomers of formoterol, about tenfold higher concentration was required to obtain the same degree of inhibition when given intratracheally as compared with administration in the external medium. There was no indication in any of the experimental approaches that (S;S)-formoterol or (S)-terbutaline might enhance the response to cholinergic or NANC-related stimuli. Chirality 8:567–573, 1996. © 1997 Wiley-Liss, Inc.

KEY WORDS: formoterol; terbutaline; enantiomers; hyperreactivity; trachea; guinea-pig

All bronchodilating β_2-adrenoceptor agonists in common clinical use are derivatives of phenylethanolamine and are available as racemates. The agonistic effect resides predominantly in the enantiomer with (R)-configuration at the carbon atom related to the alcoholic hydroxyl group. There is no apparent interaction with the less active (S)-enantiomer as demonstrated, inter alia, for terbutaline[1] and formoterol.[2] With few exceptions, all pharmacological studies on this class of compounds have been performed using racemates.

There is substantial evidence that, under some experimental conditions, *rac*-β-adrenoceptor agonists may cause airway hyperreactivity to various contractile stimuli.[3–5] In recent years the suspicion has been raised that the distomer, usually the (+)-(S)-enantiomer, is responsible for this effect.[6] Thus (+)-(S)-salbutamol, infused intravenously for 1 hour, enhanced airway reactivity to histamine in the anaesthetized guinea-pig.[7] Moreover, preincubation of guinea-pig airway smooth muscle preparations with (+)-(S)-salbutamol increased the contractile response to carbachol.[8] Against this view, repeated inhalation of (+)-(S)-salbutamol for 4 days did not change the reactivity to histamine or ovalbumin in sensitized guinea-pigs.[9]

In the present study, we have explored the ability of the distomers of terbutaline and formoterol to induce adverse effects in isolated airway smooth muscle from guinea-pig during various experimental conditions. Thus the possibility that the distomer attenuates the relaxant effect of the eutomer on continued exposure was investigated with carbachol-contracted strips from guinea-pig trachea. In other experiments the effects of the enantiomers of terbutaline and formoterol on cholinergic and excitatory non-adrenergic/non-cholinergic (e-NANC) contractions induced by electrical field-stimulation were observed. Finally, in some experiments with the enantiomers of formoterol, the possibility that the epithelium contributes to induction of cholinergic hyperreactivity was explored with a vagus nerve-trachea tube preparation.

MATERIAL AND METHODS

Animals and General Experimental Procedure

Male Dunkin-Hartley guinea-pigs (Møllegaard, Denmark), weighing 200–400 g, were anaesthetized with pentobarbitone and exsanguinated by cutting the subclavian arteries. The trachea and the main bronchi, with or without the adhering vagus

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nerves were dissected out and placed on a dissection dish containing oxygenated Krebs solution at room temperature. The solution had the following composition in mmol/l: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.16, $NaHCO_3$ 25, KH_2PO_4 1.18, and D-glucose 11.1. The material was then prepared for measurements according to one of the procedures described below. All preparations were mounted in water jacketed organ baths (40 ml) with Krebs solution at 37°C. The solution was continuously oxygenated with a stream of O_2 containing 5% CO_2.

Carbachol Contracted Strip Preparations of the Trachea

The trachea was freed from connective tissue and cut into sections comprising two cartilage rings. Surgical silk (4-0) was fastened to the cartilage on each side of the muscle. The cartilage was then cut open ventrally and the strip was mounted in the organ bath. Isometric tension was measured with a Grass force-displacement transducer (FT03C). The signals were transformed in an NB-MIO-16L-9 analogue digital converting board and registered in a Macintosh IIfx or Quadra 700 computer with a data acquisition and evaluation programme made with the LabView 2 signal-processing software (National Instruments, Austin, TX). A basal tone of 5 mN (approximately equivalent to 0.5 g load) was applied and the tissue was allowed to stabilize for 1 hour. The viability of the tracheal strips was tested by adding 0.1 μmol/l carbachol and, 15–20 min later, 2.3 μmol/l terbutaline. This was followed by a 60 min rinsing and recovery period. Preparations which did not respond with contractile and relaxant effects were discarded.

The experiment proper started with the addition of 1 μmol/l carbachol. When, after 15–20 min, a stable contraction was established, terbutaline or formoterol was added to the bath as indicated in the text. Control experiments with the vehicle were run in parallel. Sixty minutes after the addition of the test compound, 1 mmol/l theophylline was given to establish the maximum attainable relaxation. The maximum relaxing effect of the test compound and the relaxant state after 60 min of incubation were calculated as a percentage of the relaxation produced by theophylline.

Electrical Field Stimulation of Strip Preparations of Trachea and Main Bronchi

Strips, comprising three cartilage rings, were dissected out from the trachea or the main bronchi. Surgical silk (4-0) was fastened to the cartilage on each side of the muscle. The cartilage was cut open ventrally and the strip was mounted on a holder between two ring-shaped (ϕ = 5 mm) electrodes, placed 20 mm apart, and transferred to an organ bath. Isometric tension was recorded as described above. A basal tone of 10 mN was applied to the tracheal and 6 mN to the bronchial strips. After an initial check that the strips responded to electrical stimulation, the preparations were allowed to stabilize for 60 min during rinsing every 20 min. Transmural field-stimulation was induced by trains of biphasic pulses (0.5 ms, 200 mA) at 12 Hz for 20 s, delivered by a specially designed computer-controlled constant-current stimulator.[10] Each preparation was stimulated with eight trains of pulses separated by recovery periods of 20–30 min duration.

The experiment started with two control stimulations. Either enantiomer of terbutaline or formoterol was then added in a cumulative fashion as indicated in the text, each increment given 7 min before the next stimulation. After the last train of stimuli, when the tension had returned to the basal level and the drugs had been washed out, 100 μmol/l carbachol was added to establish maximum contraction. The contractile effects were expressed as a percentage of the effect of carbachol. Concentration-response curves were constructed and EC_{50}-values were estimated.

Vagus Nerve-Trachea Tube Preparation

The trachea with adherent vagus nerve was dissected out, prepared, and mounted for recording of the intratracheal pressure as described elsewhere.[11] Recordings were made on a Grass model 7D Polygraph. The nerve stumps were connected to a Grass S88 stimulator with a bipolar suction electrode. Regular contractions, resulting in pressure increase in the fluid-filled lumen, were evoked by bilateral stimulation of the vagus nerve with supramaximal pulses of 0.2 ms duration at 20 Hz for 5 s every 100 s.

Either of the enantiomers of formoterol was added in increasing doses to the external medium or into the fluid-filled lumen of the trachea tube. Time was allowed for effect equilibrium to be reached at each concentration level, usually 15–20 min. All effects were calculated as a percentage of the nerve-induced increase in intratracheal pressure recorded just before administration of the first dose of the test compound. Concentration-response curves were constructed and EC_{50}-values were calculated for each curve.

Drugs

The drugs used and their sources were: *rac*-terbutaline sulfate, (−)-(R)-terbutaline hydrobromide (99.5 ± 0.5% pure), (+)-(S)-terbutaline hydrobromide (99 ± 1% pure), *rac*-formoterol fumarate, (−)-(R;R)-formoterol fumarate (containing 1.5% of the (S;S)-enantiomer), (+)-(S;S)-formoterol fumarate (containing <0.1% of the (R;R)-enantiomer) and theophylline (Astra Draco AB, Sweden), carbamylcholine chloride (carbachol, Sigma Chemical Co., St. Louis, MO) and pentobarbitone sodium (Apoteksbolaget, Sweden). Solutions were made up in saline, when necessary with the aid of a few drops of acetic acid.

Statistics

The data are expressed as mean ± SE with the number of experiments in parenthesis. Statistical evaluation was made using a two-tailed Student's *t*-test $P < 0.05$ was considered statistically significant.

RESULTS

Maintenance of Relaxation

A single dose of *rac*-terbutaline, 200 μmol/l, caused a near maximum relaxation (about 90%) of the carbachol-contracted tracheal smooth muscle within 5 min (Fig. 1A). In the continued presence of terbutaline, the muscle recovered in tone and after 60 min almost 50% of the relaxing effect was lost ($P < 0.001$). An equieffective dose of (R)-terbutaline, 100 μmol/l, produced almost identical results. Conversely,

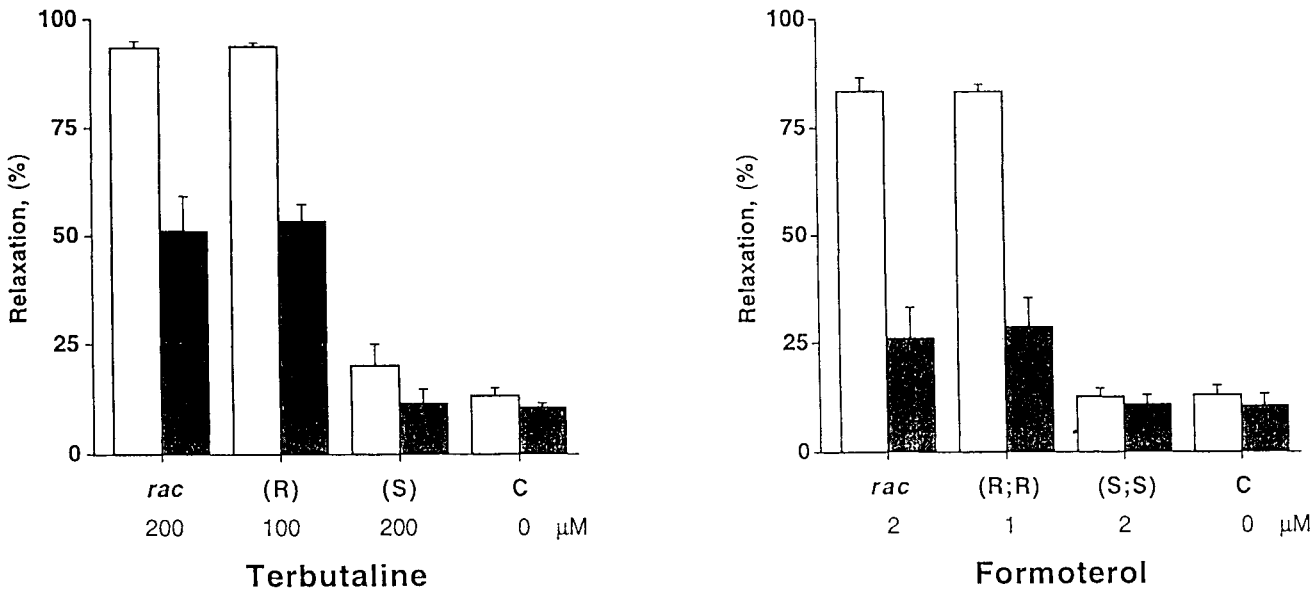

Fig. 1. Relaxation by terbutaline and formoterol of guinea-pig tracheal smooth muscle, contracted with 1 μmol/l carbachol. The racemate, either of the enantiomers or the vehicle (C) were given as indicated. The immediate maximum relaxation (open columns) and the relaxation remaining after 60 min exposure (filled columns) are shown. The data are the means ± SE of 6–7 experiments.

200 μmol/l of (S)-terbutaline caused only a marginal and, compared with the spontaneous relaxation displayed by the vehicle-treated control, not statistically significant relaxation ($P > 0.2$). There was no indication that preparations incubated with (S)-terbutaline increased in tone compared with the vehicle control upon prolonged exposure.

Very similar results were obtained with formoterol. Thus, 2 μmol/l *rac*-formoterol and 1 μmol/l (R;R)-formoterol caused an initial relaxation by about 80%, an effect which faded more than 50% in both treatment groups ($P < 0.001$) during the course of 60 min (Fig. 1B). (S;S)-formoterol, 2 μmol/l, was without any effect, relaxant or contractile, compared with the control.

Inhibition of Cholinergic and Noncholinergic Contraction

Transmural field-stimulation of the tracheal and bronchial smooth muscle induced a rapid phasic contraction (related to a cholinergic response) followed by a more slow and sustained excitatory response (related to e-NANC stimulation)[12] in the trachea, separated by a transient relaxatory component (Fig. 2). The contractile force of the first, rapid phase reached 30–40% of the maximum contraction induced by 100 μmol/l carbachol (Fig. 3). The second, slow phase was somewhat weaker with a peak response between 20 and 30% of the carbachol maximum. With no drugs added to the medium the contractions, evoked every 20 min, were reproducible for at least 3 hours. Thus the cholinergic response to the last of 8–9 trains of stimuli in the trachea was 100 ± 12% (mean ± SE; n = 6) of the initial response while the last e-NANC response was 91 ± 11%. In the main bronchi the corresponding figures were 95 ± 5% (mean ± SE; n = 3) for the cholinergic and 92 ± 7% for the e-NANC response.

(R)-Terbutaline caused a concentration-dependent inhibition of both types of contraction (Fig. 3). The pEC_{50}-value for inhibition of the cholinergic response was the same in both trachea and main bronchi (Table 1). In both tissues the excitatory NANC-response appeared to be somewhat more potently inhibited than the cholinergic response. (S)-Terbutaline was at least 1,000 times less potent than (R)-terbutaline as an inhibitor of the response to cholinergic nerve stimulation and 50% inhibition was not reached with reasonable concentrations. On the other hand, there was a more potent inhibitory effect on the NANC-induced contractions. In no case (S)-terbutaline increased the force of nerve-induced contractions. The differences in the eudismic ratios obtained for terbutaline are within the experimental error.

The results obtained with (R;R)- and (S;S)-formoterol (Fig. 4) are very similar to those observed for the enantiomers of terbutaline. The only difference is the hundredfold higher potency of formoterol as compared with terbutaline (Table 1). The eudismic ratio for formoterol is comparable with that of terbutaline or possibly somewhat lower on an average. However, the variation is within the experimental error.

Influence of the Epithelium

In the vagus nerve-trachea tube preparation, (R;R)-formoterol caused a concentration-dependent and complete inhibition of the contractions induced by stimulation of the vagus nerve when added to the external medium (Fig. 5). The pEC_{50}-value for this effect was comparable with that for inhibition of the cholinergic component of the response to field-stimulation of

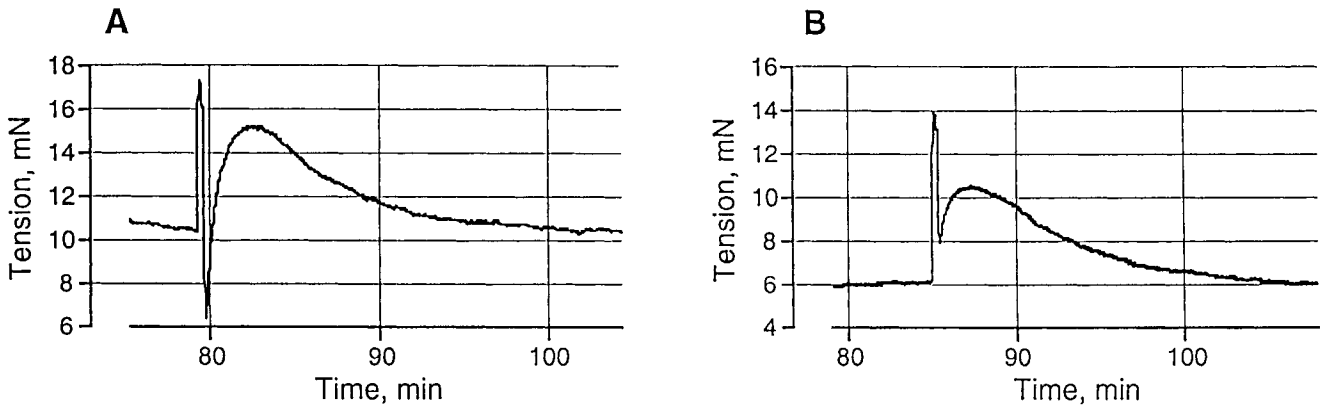

Fig. 2. The contractile response to electrical field stimulation (12 Hz for 20 s) of the trachea **(A)** or main bronchus **(B)** from guinea-pig. Recording from a single experiment.

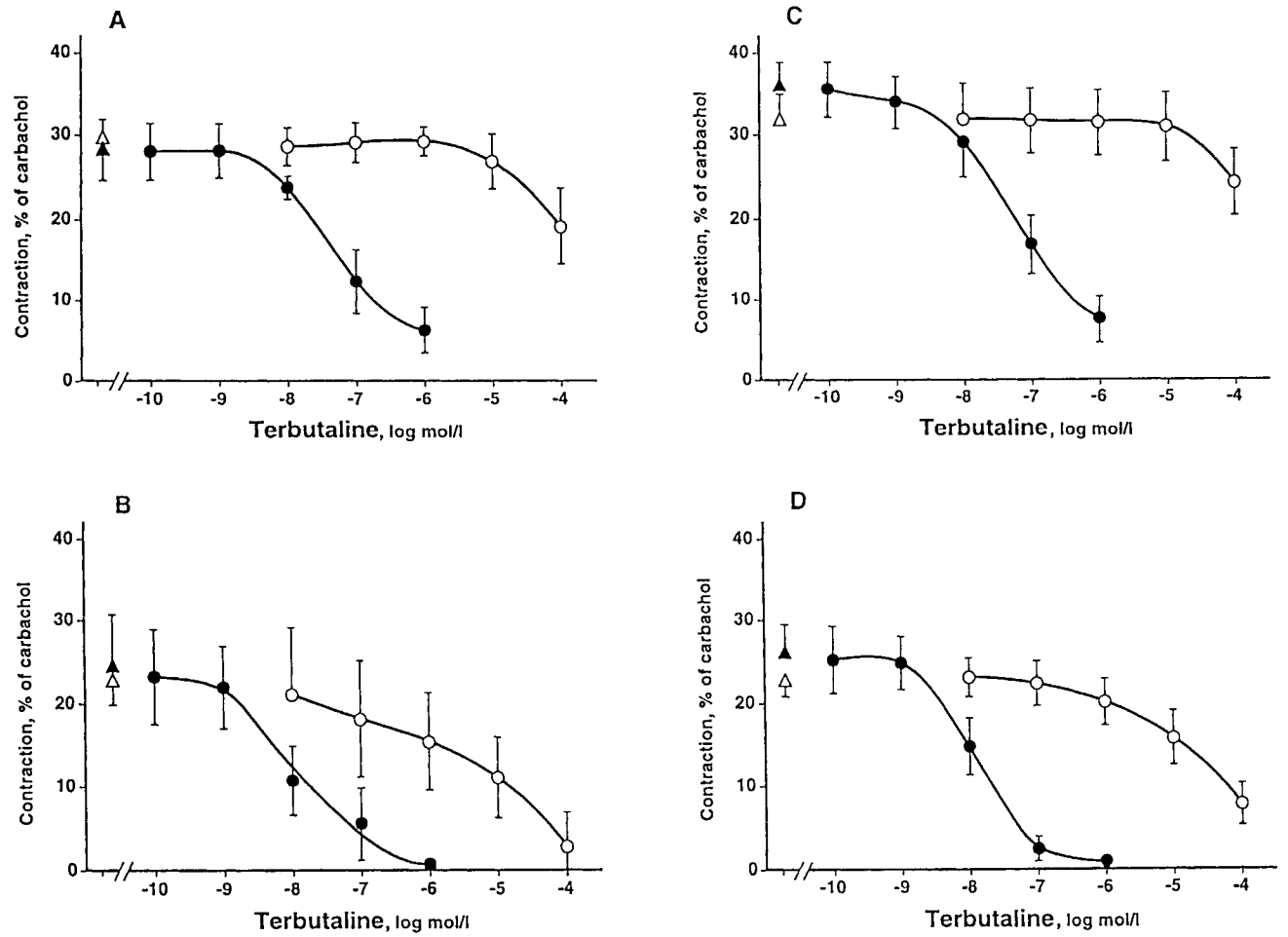

Fig. 3. Inhibition by terbutaline of contractions induced by electrical field-stimulation in strip-preparations from guinea-pig trachea **(A, B)** and main bronchi **(C, D).** Cholinergic (A and C) and e-NANC (B and D) responses were measured. (R)-terbutaline (●) and (S)-terbutaline (○) were given cumulatively. The symbols to the left (▲, △) show the contractile response before addition of terbutaline. The results are expressed as a percentage of the maximum contraction induced by 100 μmol/l carbachol. The data are the means ± SE of 5–9 experiments as indicated in Table 1.

TABLE 1. pEC_{50}-values and eudismic ratios for the inhibition by the enantiomers of terbutaline and formoterol of nerve-induced contractions of airway smooth muscle from guinea-pig[a]

	Terbutaline			Formoterol		
	pEC_{50}			pEC_{50}		
Tissue response	(R)-	(S)-	Eudismic ratio	(R;R)-	(S;S)-	Eudismic ratio
Trachea						
Cholinergic	7.13 ± 0.32 (7)	<4.0 (5)	>1,350	9.52 ± 0.24 (5)	6.44 ± 0.13 (5)	1,202
e-NANC	8.14 ± 0.33 (6)	5.27 ± 0.42 (6)	741	10.47 ± 0.17 (5)	7.56 ± 0.28 (5)	812
Main bronchus						
Cholinergic	7.15 ± 0.20 (9)	<4.0 (8)	>1,400	9.06 ± 0.17 (5)	6.20 ± 0.28 (5)	724
e-NANC	7.97 ± 0.19 (9)	4.76 ± 0.37 (8)	1,621	10.12 ± 0.16 (5)	7.42 ± 0.23 (5)	501

[a]The data are the means ± SE of (n) experiments. More details are given in Figures 3 and 4.

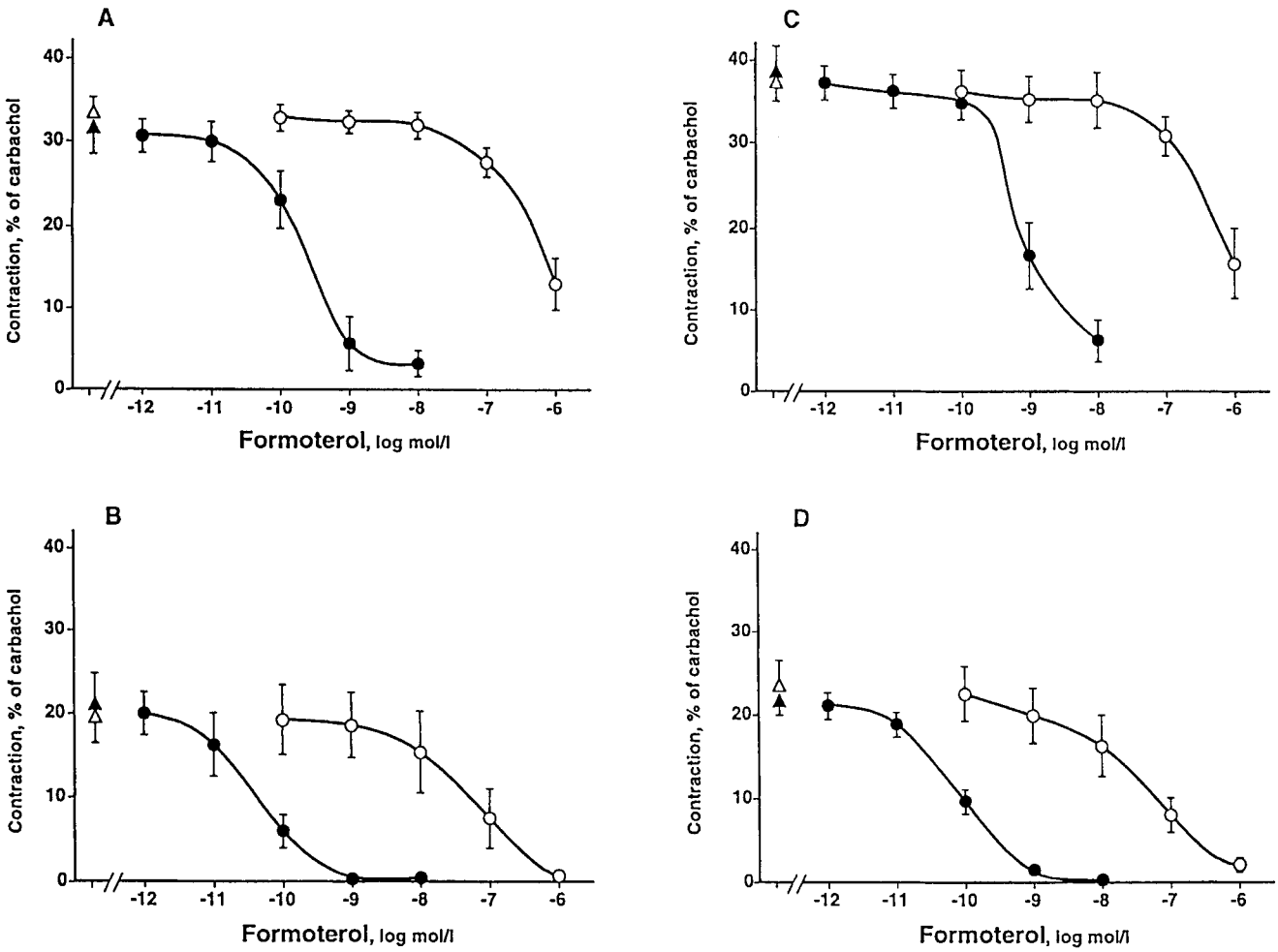

Fig. 4. Inhibition by formoterol of contractions induced by electrical field-stimulation in strip-preparations from guinea-pig trachea **(A, B)** and main bronchi **(C, D).** Cholinergic (A and C) and e-NANC (B and D) responses were measured. (R;R)-formoterol (●) or (S;S)-formoterol (○) were given cumulatively. The symbols to the left (▲, △) show the contractile response before addition of formoterol. The results are expressed as a percentage of the maximum contraction induced by 100 μmol/l carbachol. The data are the means ± SE of 5 experiments.

strip preparations of the trachea (compare Table 1 with Table 2). When (R;R)-formoterol was added to the fluid-filled lumen of the trachea, the concentration-response curve was moved about one log unit to the right ($P < 0.0001$).

(S;S)-formoterol produced qualitatively the same inhibitory effects as (R;R)-formoterol but was about 1,000 times less potent independent of the mode of administration. Thus the pEC_{50}-value obtained when (S;S)-formoterol was added intratra-

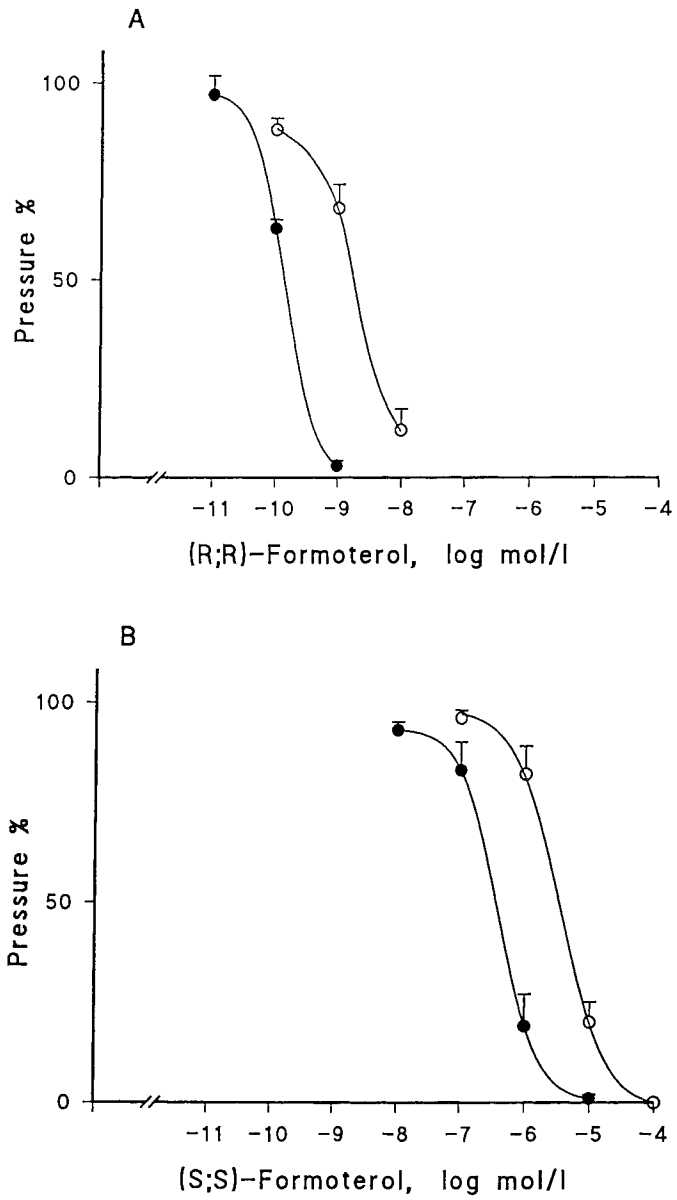

Fig. 5. Inhibition by formoterol of contractions induced by stimulation of the vagus nerve in a guinea-pig trachea-tube preparation. (R;R)-formoterol **(A)** or (S;S)-formoterol **(B)** were added cumulatively, either to the surrounding medium (●) or into the fluid-filled lumen (○). The contractile response before the addition of the test compound is normalized to 100%. The data are the means ± SE of 4–6 experiments as indicated in Table 2.

cheally was one log unit lower than when it was added to the surrounding medium ($P < 0.005$). There was no indication that (S;S)-formoterol increased the response to nerve-stimulation.

DISCUSSION

Early studies on the relaxing power of *rac*-salbutamol on carbachol-contracted tracheal smooth muscle from guinea-pig showed that, upon extended exposure, the relaxant effect waned and the muscle recovered partially in tone.[13] This phenomenon has been observed also for *rac*-formoterol[14] and here for *rac*-terbutaline as well. Recovery in carbachol-induced tone during continuous stimulation with a β_2-adrenoceptor agonist may be due to down-regulation of the β_2-adrenoceptor, up-regulation of the muscarinic receptor function, or changes in another regulatory mechanism. Our results do not exclude any of these alternatives but they clearly show that the distomers of terbutaline and formoterol are not responsible for the loss of the relaxing power. There was no sign that removal of the distomers from the racemates changed the reaction pattern, nor that the distomers per se increased the response to carbachol compared with the control.

TABLE 2. pEC_{50}-values for the inhibition by the enantiomers of formoterol of contractions induced by stimulation of the vagus nerve in the guinea-pig trachea tube preparation[a]

	Mode of administration	
Chiral form	Extratracheal	Intratracheal
(R;R)-formoterol	9.89 ± 0.06 (6)	8.65 ± 0.13 (7)
(S;S)-formoterol	6.42 ± 0.14 (5)	5.45 ± 0.15 (5)
rac-formoterol[b]	9.23 ± 0.11 (4)	8.51 ± 0.25 (4)

[a]The data are the means ± SE of (n) experiments. For more details see Figure 5.
[b]Data from Jeppsson et al.[15]

The next series of experiments was designed to elucidate possible deleterious effects of the distomers of terbutaline and formoterol on cholinergic and non-adrenergic/non-cholinergic (NANC) nerve-induced contractions in trachea and main bronchi. The eutomers, (R)-terbutaline and (R;R)-formoterol, were potent inhibitors of both types of contraction. The tenfold difference in potency observed for inhibition of cholinergic compared with e-NANC contraction may be related to the fact that with the stimulation parameters used, cholinergic contraction was stronger than the e-NANC related contraction. For the distomers of terbutaline and formoterol the inhibitory effect on nerve-induced contractions appeared only at concentrations where the inhibitory effect of the eutomers approached maximum. At concentrations below this, i.e., in the concentration range where the racemate is effective, there was no sign of hyperreactivity induced by the distomer.

The eudismic ratios for the inhibitory effect of terbutaline and formoterol obtained on electrically stimulated tracheal and bronchial strip preparations were comparable with those previously obtained on carbachol-contracted preparations from guinea-pig trachea.[1,2] The eudismic ratio for terbutaline against the e-NANC response in trachea was perhaps slightly lower than observed for this agonist in carbachol contracted preparations[1] or against the cholinergic response to nerve stimulation. If (S)-terbutaline or (S;S)-formoterol had induced an exaggerated response to excitatory (cholinergic or NANC) nerve stimulation, the eudismic ratio for the inhibitory effect should have increased compared with the relaxant effect on precontracted muscle.

In the vagus nerve-trachea tube preparation, the modulatory role of the epithelium may be studied. When added to the external medium, (R;R)- and (S;S)-formoterol generated EC_{50}-values for the inhibitory effect very similar to those obtained for the cholinergic response of the field-stimulated strip-prepa-

ration of the trachea. When formoterol was added into the fluid-filled lumen, the concentration-response curves were shifted one log unit to the right for both enantiomers. A similar shift was obtained for *rac*-formoterol,[15] thus illustrating the barrier function of the epithelium. If, however, (S;S)-formoterol caused cholinergic hyperreactivity via an epithelial factor, the dose-shift should be greater for (S;S)- than for (R;R)-formoterol. Moreover, (S;S)-formoterol at or below the threshold inhibitory concentration of 0.1 μmol/l would be expected to increase the pressure response to nerve stimulation.

The concerted evidence from these experiments in vitro shows that the distomers of terbutaline and formoterol do not induce hyperreactivity to cholinergic or e-NANC stimuli in isolated organ systems from normal animals, not even at relatively high concentrations. Moreover, there is no evidence that the distomers will attenuate the bronchodilating effect of the eutomer.[1,2] We were unable to reproduce with (S)-terbutaline or (S;S)-formoterol previous results[8] showing that preincubation of tracheal smooth muscle from guinea-pig with (S)-salbutamol enhances the contractile response to carbachol (data not shown). On the other hand, a very recent study shows that in isolated smooth muscle cells from bovine trachea (S)-salbutamol increased the carbachol-induced Ca^{2+} mobilization.[16] If these contradictory results in vitro reflect differences between the distomer of salbutamol and the distomers of terbutaline and formoterol or if other factors are involved remains to be explored.

In acute experiments on guinea-pig in vivo i.v. infusion of (S)-isoprenaline, but also (R)-isoprenaline, exaggerated the increase in airway resistance induced by bombesin.[17] This enhancing effect was prevented by vagotomy. Others found that (R)- but not (S)-isoprenaline induced hyperreactivity to histamine during similar experimental conditions.[18] More recently it was reported that the (S)-enantiomers of isoprenaline, salbutamol and terbutaline, administered via intratracheal instillation, increased the airway response to histamine in guinea-pigs sensitized to ovalbumin.[7] Proper control experiments with the (R)-enantiomers were not made, however, and it cannot be excluded that both enantiomers may, under certain experimental conditions, induce hyperreactivity as reported for isoprenaline.[17] Incomplete resolution may be a source of error in this type of experiment.[19] That the distomers exclusively, via an as yet unknown and stereoselective mechanism, should induce the hyperreactivity observed is the least probable alternative.

In another study on guinea-pigs, both normal and sensitized, an aereosol of (S)-salbutamol was inhaled for 3 min every 6 h for 4 days. No change in reactivity to histamine or allergen was observed when measured on day 5.[9] Also, in an acute study in mild asthmatics, inhalation of (S)-salbutamol did not seem to cause a clinically significant change in the response to metacholine.[20] Thus there is evidence both for and against a role of the distomers of *rac*-β_2-adrenoceptor agonists in the induction of airway hyperreactivity. It remains to be established whether the noxious effects observed under some experimental conditions are specific for the distomer, general for all members of this class of drugs and, eventually, constitute a clinical problem.

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