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# Bronchodilating properties of the VIP receptor agonist Ro 25-1553 compared to those of formoterol on the guinea-pig isolated trachea

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**Abstract**

Ro 25-1553 is a 31-amino acid analogue of vasoactive intestinal peptide (VIP) and has recently been shown to be highly selective for the $VPAC_2$-receptor. The bronchodilating property of this compound was evaluated in vitro on preparations of guinea-pig trachea, with the long-acting $\beta_2$-adrenoceptor selective agonist, formoterol, as a reference. In strip-preparations precontracted with carbachol, Ro 25-1553 caused a concentration-dependent and complete relaxation of the tracheal smooth muscle. Ro 25-1553 was 3–7 times less potent than formoterol on a molar basis, but the efficacy was comparable with that of formoterol. Both compounds showed a rapid onset of action and a similar durability of effect. Ro 25-1553 appeared to interact with formoterol as well as with salmeterol in an additive way. In vagus nerve-trachea tube preparations, when added to the external medium, Ro 25-1553 concentration-dependently and completely inhibited nerve-induced contractions. This occurred in the same concentration range as needed for relaxation of precontracted strips. Ro 25-1553 was active also when administered into the tracheal lumen albeit the concentration had to be increased. The present study supports and extends previous results suggesting that Ro 25-1553 may be a powerful alternative to the $\beta_2$-adrenoceptor agonists which prevail today. 

*Keywords:* Ro 25-1553; Formoterol; Salmeterol; Bronchodilator; Trachea; (Guinea-pig); In vitro

## 1. Introduction

Ro 25-1553 is a 31-amino acid analogue of vasoactive intestinal peptide (VIP) with the amino acid sequence:

Ac-His-Ser-Asp-Ala-Val-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-
Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-$NH_2$

where Ac = $N^{\alpha}$-acetyl (Bolin et al., 1995). Like VIP it has a bronchodilating effect in vitro as well as in vivo but it is more potent and metabolically more stable than the native compound (O'Donnell et al., 1994a). Moreover, Ro 25-1553 has the capacity to inhibit allergen-induced bronchoconstriction and eosinophil influx in the guinea-pig lung (O'Donnell et al., 1994b). Ro 25-1553, like native VIP and the $\beta_2$-adrenoceptor agonist salbutamol, increases the formation of cyclic AMP as shown in murine splenocytes (Tang et al., 1995). Recently, Ro 25-1553 has been found to be highly selective for the $VIP_2$ (now $VPAC_2$) receptor subclass (Gourlet et al., 1997), and hence designated by the International Union of Pharmacology as a tool for classification of receptors for VIP (Harmar et al., 1998).

We now report on extended studies exploring the relative potency and efficacy of Ro 25-1553 compared to the long-acting $\beta_2$-adrenoceptor agonist, formoterol. The aims of the investigation were to, (a) estimate the capability to relax strongly contracted airway smooth muscle strips, (b) measure the rate of onset of action and the durability of the relaxing effect, (c) investigate the interaction between Ro 25-1553 and the $\beta_2$-adrenoceptor agonists, formoterol and salmeterol, and (d) explore, with the aid of a vagus nerve-trachea tube preparation, the ability of Ro 25-1553 to pass the airway epithelium. All experiments were carried out on isolated preparations of guinea-pig trachea.

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## 2. Materials and methods

### 2.1. Animals and organ baths

Male Dunkin–Hartley guinea-pigs (Charles River, Sweden), 200–400 g, were anaesthetized with pentobarbitone and exsanguinated by cutting the subclavian arteries. The trachea, with or without the adhering vagus nerves was dissected out and prepared for measurements as described below. All experiments were performed in water-jacketed organ baths (40 ml) containing oxygenated Krebs solution at 37 °C. The Krebs solution had the following composition in mmol $l^{-1}$: 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 oxygenated with a mixture of 5% $CO_2$ in $O_2$.

### 2.2. Trachea strip preparation

The trachea was freed from connective tissue and cut into sections comprising two cartilage rings. Silk threads were fastened to the cartilage on each side of the muscle. The cartilage was then cut open ventrally and the strip was mounted in an organ bath. Isometric tension was measured with a Grass force transducer (FTO3). The signals were transformed in an NB-MIO-16L-9 analogue digital converting board and registered in a Macintosh Quadra 700 computer with a data acquisition and evaluation programme made with LabView 2 signal-processing software (National Instruments, Austin, TX). The preparation, mounted at a basal tone of 5 mN, was allowed to stabilize for 1 h. The viability of the preparation was tested by adding 0.1 μmol $l^{-1}$ carbachol to the bath followed, 15–20 min later, by 3 μmol $l^{-1}$ terbutaline. This was followed by a 60-min rinsing and recovery period. Preparations which did not respond with contraction to carbachol and relaxation to terbutaline were discarded.

The experiments started with the addition of carbachol. When, after 15–20 min, a stable contraction was established, the test substance was added either cumulatively or as a single dose depending on the experiment. In the interactive experiments with salmeterol, this compound or the vehicle was added 60 min before the cumulative administration of Ro 25-1553 or formoterol. After the final dose of the test compound, maximum relaxation was established by adding 10 μmol $l^{-1}$ isoprenaline followed by 1 mmol $l^{-1}$ theophylline. Additional relaxation by theophylline on the top of isoprenaline was regarded as a non-β-adrenoceptor mediated effect. Relaxant effects were calculated as a percentage of the maximum relaxation induced by theophylline added at the end of the experiment.

Table 1
Bronchodilating capacity of Ro 25-1553 compared to formoterol with respect to relaxation of tracheal smooth muscle strips precontracted with carbachol and inhibition of contractions induced by vagus nerve stimulation in a tube preparation of the guinea-pig trachea

| Contraction induced by | Ro 25-1553 | Formoterol |
|---|---|---|
| *Carbachol* | | |
| 0.1 μmol $l^{-1}$ | 8.57 ± 0.06 (5) | 9.24 ± 0.17 (5) |
| 1.0 μmol $l^{-1}$ | 7.69 ± 0.10 (6) | 8.52 ± 0.06 (6) |
| Difference statistically significant | $P < 0.001$ | $P < 0.005$ |
| *Nerve stimulation* | | |
| Bronchodilator given | | |
| Extraluminally | 8.31 ± 0.17 (6) | 9.23 ± 0.11 (4)[a] |
| Intraluminally | 6.32 ± 0.20 (6) | 8.51 ± 0.25 (4)[a] |
| Difference statistically significant | $P < 0.001$ | $P < 0.05$ |

Shown are the $pD_2$-values ± S.E. and the number of experiments. Full concentration–response curves are shown in Figs. 1 and 5.
[a] Data from Jeppsson et al. (1989a).

### 2.3. Vagus nerve-trachea tube preparation

The trachea with the adherent vagus nerves was dissected out and prepared for recording of intratracheal pressure as described elsewhere (Widmark and Waldeck, 1986). Recordings were made on a Grass model 7D Poly-

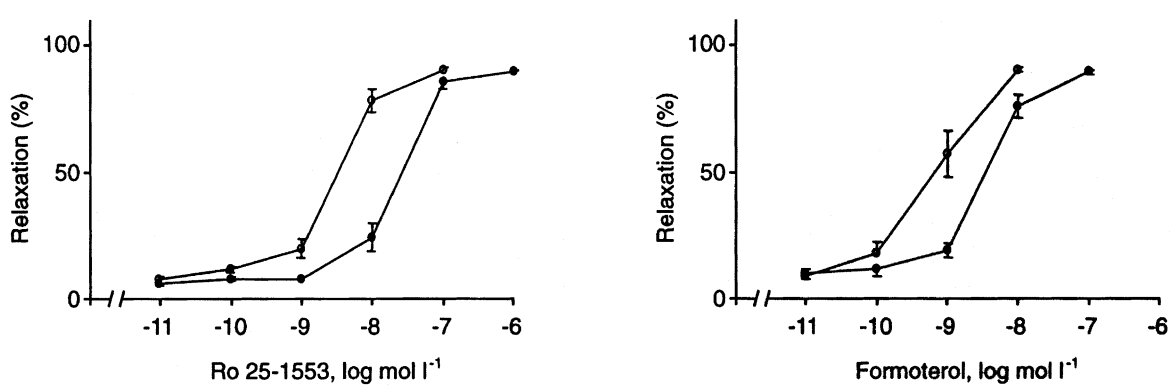


Fig. 1. Relaxation by Ro 25-1553 (left block) and formoterol (right block) of airway smooth muscle strip preparation from guinea-pig trachea. Contraction was induced by carbachol, 0.1 μmol $l^{-1}$ (open circles, $n = 5$) or 1 μmol $l^{-1}$ (closed circles, $n = 6$) before the cumulative addition of the test compounds. The results are expressed in percent of the maximum relaxation induced by 1 mmol $l^{-1}$ theophylline. Means ± S.E. are shown.

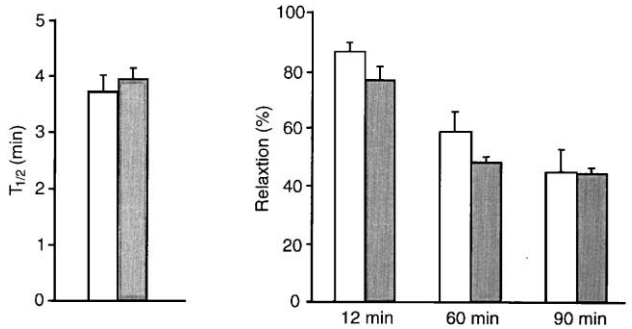


Fig. 2. Onset and durability of the relaxation induced by Ro 25-1553 and formoterol in guinea-pig trachea strip preparation. Precontraction was induced by 1 μmol $l^{-1}$ carbachol. The time to half maximum relaxation ($T_{1/2}$, left block), the maximum relaxation (in percent of the maximum relaxation by 1 mmol $l^{-1}$ theophylline, right block) recorded within 12 min and the relaxation maintained 60 and 90 min after the addition of 100 nmol $l^{-1}$ Ro 25-1553 (open bars) or 20 nmol $l^{-1}$ formoterol (shaded bars) are shown. The means ± S.E. of eight experiments are shown.

graph. The nerve stumps were connected to a Grass S88 stimulator via a bipolar suction electrode. Contractions resulting in a pressure increase in the fluid-filled lumen were evoked by bilateral stimulation of the vagus nerve at 20 Hz for 5 s every 100 s. A concentration–response curve for the test compound was obtained by adding the drug cumulatively to the external medium or to the fluid-filled lumen of the trachea tube. Time was allowed for effect equilibrium to be reached at each concentration level. All effects were calculated as a percentage of the nerve-induced increase in intratracheal pressure just before administration of the first dose of the test compound.

### 2.4. Drugs

The drugs used were: Ro 25-1553 (Roche Pharmaceuticals, Nutley, NJ), *rac*-formoterol fumarate, *rac*-salmeterol free base and theophylline (AstraZeneca R&D Lund, Sweden); carbamylcholine chloride (carbachol) and (−)-isoprenaline hydrochloride (Sigma), and pentobarbitone sodium (Apoteksbolaget, Sweden). The test compounds were dissolved in water and the dilutions were made up in saline or Krebs solution.

### 2.5. Calculations

Concentration–response curves were constructed and $EC_{50}$-values were calculated for each curve. Data are expressed as mean ± S.E. Each preparation was used for one experiment only. Statistical evaluation was done using Student's *t*-test.

## 3. Results

### 3.1. Potency and efficacy

Ro 25-1553 caused a concentration-dependent and complete relaxation of the guinea-pig trachea strip preparation contracted with 0.1 μmol $l^{-1}$ carbachol (Fig. 1). A further increase in smooth muscle tone achieved by increasing the concentration of carbachol to 1 μmol $l^{-1}$ resulted in a sevenfold shift of the concentration–response curve to the right ($P < 0.001$) but with maximum relaxation maintained. Very similar results were obtained with formoterol (Fig. 1). With the low level of carbachol-induced tone, Ro 25-1553 was five times less potent than formoterol on a molar base ($P < 0.01$; Table 1). When compared at the high level of tone, Ro 25-1553 was seven times less potent than formoterol ($P < 0.001$). It should be noted that the slopes of the concentration–response curves were also similar for the two compounds.

### 3.2. Onset and durability of action

The next experiment aimed at estimation of onset of action and durability of effect. When added to a strip-pre-

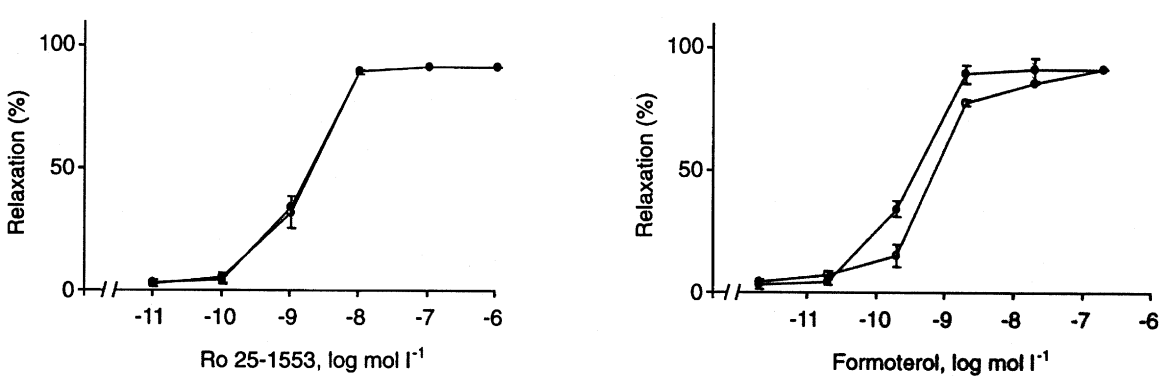


Fig. 3. The interaction between Ro 25-1553 and formoterol on the guinea-pig trachea strip preparation. Precontraction was induced by 0.1 μmol $l^{-1}$ carbachol. Ro 25-1553 and formoterol were added cumulatively, either separately (open circles) or in a constant ratio 5:1 mixture (closed circles, identical data in both blocks). The means ± S.E. of eight experiments are shown.

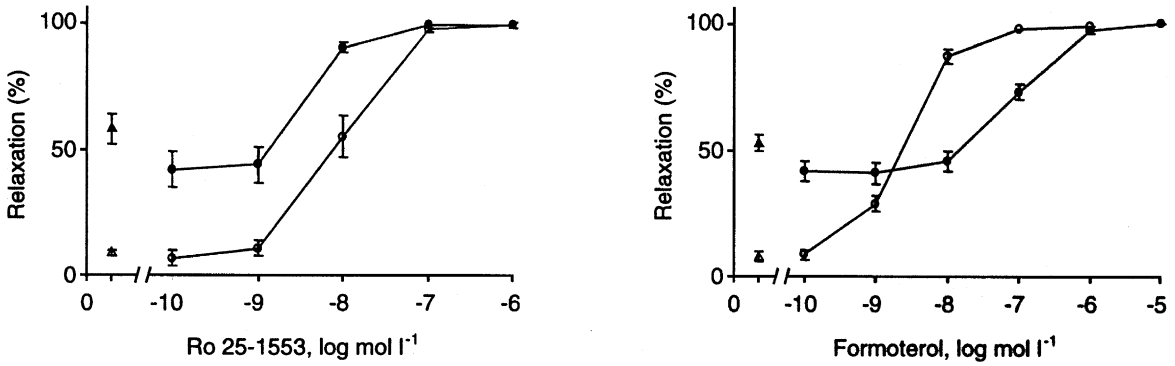


Fig. 4. The interaction between Ro 25-1553 and salmeterol compared to the interaction between formoterol and salmeterol in relaxing strip-preparations from guinea-pig trachea. In all treatment groups, precontraction was induced by 1 μmol $l^{-1}$ carbachol which was present in the organ bath throughout the experiment. Salmeterol, 1 μmol $l^{-1}$, or the vehicle were added before the cumulative addition of Ro 25-1553 (left block) or formoterol (right block), with (closed circles) or without (open circles) salmeterol. The effect of salmeterol alone (closed triangles) or the vehicle (open triangles) before adding Ro 25-1553 or formoterol is indicated to the left in each block. The means ± S.E. of six experiments are shown.

paration of the trachea, precontracted with 1 μmol $l^{-1}$ carbachol, half-maximum relaxation by 100 nmol $l^{-1}$ Ro 25-1553 (producing approximately an 80% relaxation) was reached in about 4 min. This rate of onset was comparable with that obtained with an equieffective concentration of formoterol, 20 nmol $l^{-1}$ (Fig. 2). Maximum relaxation to the selected doses was about 80% of the maximum relaxation by theophylline and was reached within 12 min. In the continued presence of Ro 25-1553, the relaxing power was not maintained and the preparation recovered about half of its tone within an hour and a half ($P < 0.001$), a pattern which closely resembled that displayed by formoterol (Fig. 2).

### 3.3. Interaction with formoterol

A concentration–response curve was obtained for a 5:1 mixture (on a molar basis) of Ro 25-1553 and formoterol on a strip preparation of the trachea, precontracted with 0.1 μmol $l^{-1}$ carbachol. The 5:1 ratio was deduced from the potency relationship obtained in the experiments presented above, assuming that equieffective concentrations of the two drugs are given along the whole concentration–response curve. The mixture produced a concentration–response curve superimposable on that produced by Ro 25-1553 given alone (Fig. 3). When compared to formoterol, however, the mixture was twice as potent as formoterol given alone ($P < 0.01$).

### 3.4. Interaction with salmeterol

In strip preparations precontracted with 1 μmol $l^{-1}$ carbachol, the maximum relaxation caused by 1 μmol $l^{-1}$ salmeterol, given in the beginning of the experiment, was about 50%. Cumulative addition of Ro 25-1553 caused a further relaxation up to 100% (Fig. 4). The potency of Ro 25-1553 was not affected by salmeterol as compared with the vehicle-controlled preparation given Ro 25-1552 alone. Also formoterol produced a further relaxation on the top of salmeterol, but the concentration–effect curve for formoterol was shifted about one and a half log units to the right by salmeterol (Fig. 4).

### 3.5. Transepithelial access

Ro 25-1553 also inhibited, concentration-dependently and completely, contractions induced by electrical stimulation of the vagus nerve in the trachea tube preparation (Fig. 5). When the test compound was added to the external medium, the $pD_2$-value for this inhibitory effect of Ro 25-1553 was similar to that obtained for the relaxatory effect on strip-preparation precontracted with carbachol, 0.1 μmol $l^{-1}$ (Table 1). Intraluminal administration

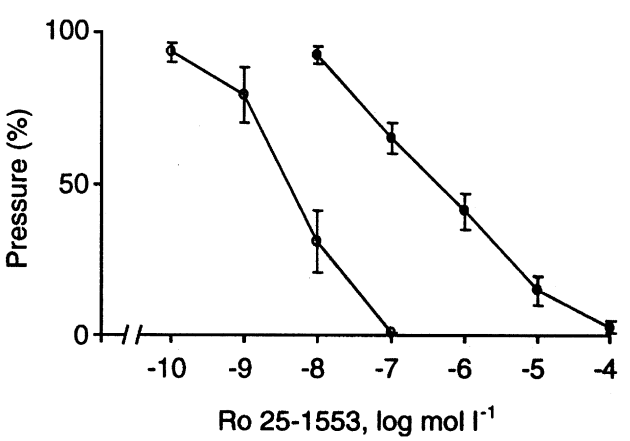


Fig. 5. Inhibition by Ro 25-1553 of contractions of the tube-preparation of guinea-pig trachea, induced by stimulation of the vagus nerve. Contractions were measured as increase in intraluminal pressure. Ro 25-1553 was added in a cumulative manner either to the external medium ( open circles) or into the fluid-filled lumen (closed circles). The means ± S.E. of six experiments are shown.

of Ro 25-1553 resulted in a hundredfold rightward shift of the concentration–response curve ($P < 0.001$) and maximum inhibition was achieved only at 100 μmol $l^{-1}$ (Fig. 5).

## 4. Discussion

The present data confirm and extend previous results (O'Donnell et al., 1994a) which show that the VIP receptor agonist Ro 25-1553 potently relaxes airway smooth muscle with an $EC_{50}$ in the nanomolar range. Our data further show that Ro 25-1553 is 3–7 times less potent than formoterol on a molar base. For both compounds, the concentration–response curves were moved to the right with no depression of the maximum relaxation when the degree of precontraction was increased by carbachol. This expression of functional antagonism is in line with a bronchodilator with high efficacy (Buckner and Saini, 1975). Thus, Ro 25-1553, like formoterol, has a considerably higher efficacy than salmeterol according to the present and previous (Jeppsson et al., 1992) data.

The onset of action of Ro 25-1553 was comparable to that displayed by formoterol, i.e. it is considerably faster than that of salmeterol and more comparable to salbutamol (Jeppsson et al., 1989a). However, the relaxed state was not maintained over time for either Ro 25-1553 or formoterol. In the continued presence of a $\beta_2$-adrenoceptor agonist, tracheal smooth muscle strongly contracted by carbachol partially recovers in tone over time. This phenomenon was originally shown for salbutamol (Raper and Malta, 1973) but has been demonstrated for terbutaline and formoterol as well (Källström et al., 1996). It has been interpreted as a balance between desensitization and resensitization processes at the $\beta_2$-adrenoceptor (Wachsman et al., 1997). The tachyphylaxis to Ro 25-1553 developed under the harsh experimental conditions in vitro used here, i.e. near maximum contraction by carbachol, may have a similar explanation. It is uncertain whether these results extend to in vivo conditions.

The combined effect of Ro 25-1553 and formoterol, given together in approximately equieffective doses, did not differ from the relaxing effect of Ro 25-1553 given alone. Compared to formoterol, the mixture was about twice as potent. Thus, there is a synergism between Ro 25-1553 and formoterol which is at most additive. Since the two compounds appear to act through the same second messenger, cyclic AMP (Tang et al., 1995), this outcome is expected. No negative interaction was observed.

It has previously been shown (Jeppsson et al., 1992), and now reconfirmed, that being a partial agonist at $\beta_2$-adrenoceptors, salmeterol inhibits the relaxing effect of formoterol on strongly contracted airway smooth muscle in a competitive way. Contrary to formoterol, however, the relaxing power of Ro 25-1553 was not attenuated by salmeterol. This shows that the VIP receptor agonist Ro 25-1553, operating at a different receptor system, has a relaxing effect additive to and independent of that of salmeterol. Thus, Ro 25-1553 may bring effective bronchodilation in patients who have developed tolerance after taking high doses of $\beta_2$-adrenoceptor agonists or when the $\beta_2$-adrenoceptors have been blocked by inappropriate intake of a β-adrenoceptor antagonist.

Ro 25-1553 inhibited the contractions of the guinea-pig trachea tube preparation evoked by stimulation of the vagus nerve. The vagal response in this preparation is entirely cholinergic (Widmark and Waldeck, 1986). The inhibitory effect occurred in the same concentration range as relaxation of precontracted muscle strips. This is again a property shared with formoterol (Jeppsson et al., 1989a) and a number of other β-adrenoceptor agonists as shown in Fig. 6. While the inhibitory action of Ro 25-1553 in the nerve trachea-tube preparation is most probably an effect at the smooth muscle level, it cannot be excluded that part of the inhibition is a presynaptic event as has been suggested for native VIP (Martin et al., 1990).

When Ro 25-1553 was given into the tracheal lumen, a hundredfold higher concentration was needed to reach the same degree of inhibition compared to extraluminal administration. This is to be expected for a hydrophilic compound since the epithelium acts as a diffusion barrier. Similar results have been obtained with the hydrophilic $\beta_2$-adrenoceptor agonists, terbutaline and salbutamol, which are both active after local administration (Jeppsson et al., 1989b). For endogenous VIP, metabolic degradation of the peptide in the epithelium may contribute to the poor effect seen after intratracheal administration (Takubo et al., 1991). To what extent Ro 25-1553 is subject to degradation in the epithelium is unknown. But the transepithelial

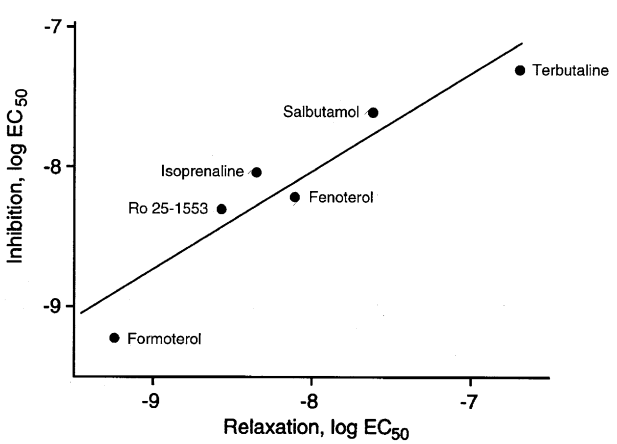


Fig. 6. Relaxation of carbachol (0.1 μmol $l^{-1}$) induced tone versus inhibition of nerve-induced (cholinergic) contractions in the guinea-pig trachea. The $pEC_{50}$-values for relaxation of precontracted strip-preparations are plotted against the $pEC_{50}$-values for inhibition of vagus nerve-induced contractions in a tube-preparation. The correlation coefficient for the regression line, $r = 0.94$. Data on Ro 25-1553 from Table 1 together with historical data on β-agonists from this laboratory (Jeppsson et al., 1989a,b).

passage of this large molecule is sufficient for the compound to exert maximum inhibition of contraction.

In conclusion, Ro 25-1553 is a potent and efficient bronchodilator, in efficacy comparable to formoterol but acting via a different receptor system. The discovery that Ro 25-1553 has a high degree of selectivity for $VPAC_2$ receptors adds to the development potential of the drug. The present study supports previous results suggesting that Ro 25-1553 or an analogue thereof may be a powerful alternative to the $\beta_2$-adrenoceptor agonists which prevail today.

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