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Tracheal relaxing effects and β_2 adrenoceptor selectivity of S1319, a novel sponge-derived bronchodilator agent, in isolated guinea-pig tissues

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1 S1319 (4-hydroxy-7-[1-(1-hydroxy-2-methylamino)ethyl]-1,3-benzothiazol-2(3*H*)-one acetate), a novel non-catecholamine β-adrenoceptor agonist, has been compared with isoprenaline, salbutamol and formoterol for activity *in vitro* on a range of β-adrenoceptor containing preparations from guinea-pig.

2 S1319, like isoprenaline, salbutamol and formoterol, relaxed preparations of guinea-pig trachea (contracted by histamine) in a concentration-dependent manner. The relaxing activity of S1319 appeared to be more potent than that of isoprenaline and salbutamol, and similar to that of formoterol (pD_2 values of 10.58 ± 0.03 vs 7.60 ± 0.01, 7.50 ± 0.01 and 10.52 ± 0.04, respectively), and was blocked by the β_2-adrenoceptor selective antagonist (ICI 118,551). The intrinsic activity of S1319 was close to 1.0.

3 In the β_1-adrenoceptor containing preparations, guinea-pig right and left atria, a monophasic inotropic response of S1319 was observed. The pD_2 value of S1319 for left atrial and right atrial inotropism was 6.70 ± 0.15 and 7.81 ± 0.01, respectively.

4 The selectivity ratio (trachea/left atrial inotropism) of S1319, formoterol, salbutamol and isoprenaline was 8523, 284, 4.8 and 0.45, respectively. The relative selectivity ratio of S1319 was 18743, 1858 and 30 times greater than that of isoprenaline, salbutamol and formoterol, respectively.

5 Relaxant responses of guinea-pig trachea to S1319 declined rapidly when the agonist was washed from the tissues, with complete recovery within 30 min. The duration of action of S1319 was similar to that of isoprenaline and less than that of salbutamol and formoterol.

6 In summary, S1319, a sponge-derived β-adrenoceptor agonist, is a potent and selective β_2-adrenoceptor agonist with a short-duration of action in isolated guinea-pig tracheas.

Keywords: β_2-Adrenoceptor agonist; tracheal smooth muscle relaxation; duration of action

Abbreviations: ICI 118,551, erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol; pD_1, negative logarithm of ED_{50}; S1319, 4-hydroxy-7-[1-(1-hydroxy-2-methylamino)ethyl]-1,3-benzothiazol-2(3*H*)-one acetate.

Introduction

β_2-Adrenoceptor agonists form an important class of therapeutic agents in asthma. Selective β_2-adrenoceptor agonists are clinically the most widely used and effective bronchodilators because they have low cardiac side effects. Historically, isoprenaline has been the most popular β-adrenoceptor stimulant but it has some disadvantages such as tachycardia due to its low selectivity towards the airway. Consequently, many bronchodilators such as trimetoquinol (Sato *et al*., 1980), salbutamol (O'Donnell, 1972; Cullum *et al*., 1969), procaterol (Yabuuchi, 1977), formoterol (Ida, 1976), salmeterol (Ball *et al*., 1987a,b; 1991; Bradshaw *et al*., 1987), TA-2005 (Kikkawa *et al*., 1991; Voss *et al*., 1992; 1994) which are selective for bronchial smooth muscle, have been developed.

Recently, S1319 (4-hydroxy-7-[1-(1-hydroxy-2-methylamino)ethyl]-1,3-benzothiazol-2(3*H*)-one acetate, Figure 1) has been isolated from marine sponge as a novel bronchodilating agent. Although S1319 does not have a catechol moiety, pharmacological studies have revealed that S1319 possesses binding affinity for β_2-adrenoceptor (Suzuki *et al*., 1999).

In the present study, we investigated the tracheal relaxing effect and β_2-selectivity of S1319 in guinea-pigs by pharmacological studies to characterize its properties. The effects of S1319 were also compared with those of other β-adrenoceptor agonists, salbutamol, formoterol and isoprenaline. It was found that S1319 exhibited much more β_2-selectivity than the other compounds tested and had a short duration of action.

Methods

Experiments using isolated guinea-pig trachea

Male Hartley guinea-pigs (250–700 g, Charlesriver Japan Inc., Atsugi, Japan) were killed by exsanguination following a blow to the head. The trachea was dissected. Tracheal strips were prepared and mounted in a 10 ml organ bath filled with Tyrode's solution (in mM): NaCl 137.0, $NaHCO_3$ 11.9, KCl 2.68, $CaCl_2$ 1.89, $MgCl_2$ 1.09, NaH_2PO_4 0.24 and glucose 5.6, and then continuously gassed with 95% O_2 5% CO_2 and

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maintained at 37°C. Test compounds were added cumulatively in the tonic phase of the contraction. Tension changes of the preparation were recorded isometrically with a strain gauge transducer (TB-611T, Nihon Kohden, Tokyo, Japan) on an ink-writing recorder (AP-621G, Nihon Kohden). The preparation was stretched to a resting tension of 0.5 g and was allowed to equilibrate for an hour. The guinea-pig tracheal preparations were precontracted with 1×10^{-5} M histamine and the magnitude of each response was measured and calculated as a percentage of the maximum isoprenaline (3×10^{-6} M) response obtained in the final control curve. Agonistic activities of the compounds were estimated by pD_2 (negative log molar concentration that produced 50% relaxation) obtained from each concentration-response curve and by their intrinsic activity.

To compare the duration of action of β-agonists, a submaximal relaxation of trachea was induced with methacholine (1×10^{-5} M), which induced a continuous contraction. β-agonists were tested at a single concentration sufficient to cause maximal relaxation of the trachea. The selected concentrations were: S1319 (1×10^{-8} M), formoterol (1×10^{-8} M), salbutamol (3×10^{-7} M), and isoprenaline (3×10^{-7} M). At time 0, β-agonists were added to the bathing solution inducing relaxation and immediately the washing fluid removed by a single change of bathing solution. At intervals thereafter, the decline in relaxation relative to the precontraction level was measured.

Guinea-pig atria

Separate preparations of guinea-pig right and left atria were suspended in 30 ml tissue baths containing Krebs Henseleit solution maintained at 37°C and equilibrated with 95% O_2 and 5% CO_2 for 30–60 min. The beating rate of the atria was measured with a cardiotachometer (AT-601T, Nihon Kohden, Tokyo, Japan) triggered by contractions which were measured isometrically by a force-displacement transducer (TB-611T, Nihon Kohden).

The left atrium was driven with square-wave pulses of about 30% above the threshold voltage and 5 ms duration at a rate of 2 Hz, delivered by an electronic stimulator through bipolar platinum electrodes, essentially the same as those described by Blinks & Koch-Weser (1961). Contractile force was measured isometrically as with the right atria. The resting tension was 0.5 g for each preparation. Recordings were made on an ink-writing recorder. Test compounds were also added cumulatively in the tissue experiment.

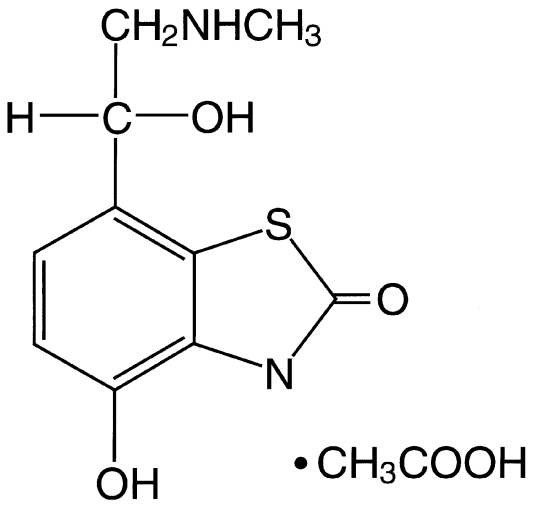

Figure 1 Chemical structure of S1319.

Materials

S1319 acetate was synthesized in our laboratory. Other drugs used were as follows: (−)-isoprenaline hydrochloride, salbutamol sulphate (Research Biochemicals, Inc., Natick, MA, U.S.A.), histamine hydrochloride and acetylcholine (Sigma Chemical Co., St. Louis, MO, U.S.A.), ICI 118,551 (Tocris Cookson Ltd., Bristol, U.K.), and formoterol fumarate (Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan). All other chemicals used were of reagent grade.

Statistics and calculations

Mean pD_2 values are given together with the standard error of the mean (s.e.mean). pD_2 values were obtained by Kaleida Graph (Synergy Software, PA, U.S.A).

Results

Effect of S1319 on contraction in the isolated guinea-pig trachea

To examine the potency of S1319 (Figure 1) to relax histamine-induced contraction of guinea-pig trachea, a comparison was made with formoterol, salbutamol and isoprenaline. The guinea-pig tracheal preparations were precontracted with 1×10^{-5} M histamine. S1319 concentration-dependently relaxed the histamine-induced contraction of isolated trachea at a 1×10^{-11} M or higher concentration (Figure 2). The concentration-response curve for S1319 ran parallel to those of isoprenaline, salbutamol and formoterol. The negative logarithm of the ED_{50} ($pD_2 \pm$ s.e.mean) value of S1319, formoterol, salbutamol and isoprenaline was 10.58 ± 0.03 ($n=6$), 10.52 ± 0.04 ($n=4$), 7.50 ± 0.01 ($n=4$) and 7.60 ± 0.01 ($n=6$), respectively (Table 1). From the pD_2 value, the relaxing effect of S1319 was found to be 955, 1202 and 1.1 times as

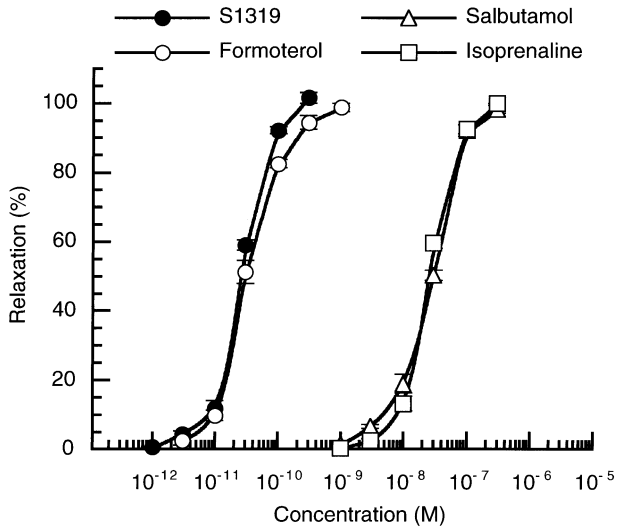

Figure 2 Concentration-response curves for S1319, isoprenaline, salbutamol and formoterol obtained in tracheal preparations isolated from guinea-pigs as a percentage of the maximal response for isoprenaline. Precontraction was induced by 1×10^{-5} M histamine. Each point represents the mean of 4–6 preparations. Vertical bars represent the s.e.mean.

potent as that of isoprenaline, salbutamol and formoterol, respectively. The intrinsic activity of all compounds was close to 1.0.

Influence of β-adrenoceptor antagonist on the relaxing effect of S1319

The interaction between β-adrenoceptor antagonists and S1319 was determined on histamine-contracted guinea-pig tracheal preparations. The non-selective β-adrenoceptor antagonist propranolol (data not shown) and β_2-adrenoceptor selective antagonist ICI 118,551 (Figure 3) produced a concentration dependent inhibition of S1319-induced relaxation, resulting in a shift to the right of the concentration-response curve.

Effect of S1319 on isolated guinea-pig atria

Table 1 pD_2 and intrinsic activities of S1319, formoterol, salbutamol and isoprenaline for β-adrenoceptors in isolated tracheal and atrial preparations of guinea-pig

	pD_2*			
	Trachea	*Left atrium*	*Right atrium*	
Agonists	*relaxation*	*inotropism*	*Chronotropism*	*Inotropism*
S1319	10.58±0.03	6.07±0.15	10.47±0.19	7.81±0.01
	(955)†,†	(0.01)	(603)	(0.4)
α†	1.00±0.00	0.51±0.01	1.00±0.00	0.60±0.05
Formoterol	10.52±0.04	8.07±0.05	9.53±0.03	8.14±0.04
	(832)	(1.4)	(69)	(0.9)
α	0.99±0.01	1.00±0.00	1.00±0.00	1.00±0.00
Salbutamol	7.50±0.01	6.82±0.04	7.74±0.01	7.00±0.01
	(0.8)	(0.8)	(1.1)	(0.07)
α	0.98±0.01	0.80±0.00	0.89±0.00	0.83±0.01
Isoprenaline	7.60±0.01	7.94±0.02	7.69±0.03	8.16±0.01
	(1)	(1)	(1)	(1)
α	1.0	1.0	1.0	1.0

Values are given as the mean±s.e.mean. ($n=4-6$) *pD_2 = negative logarithmic molar concentration for 50% maximum response. †α = intrinsic activity; maximum response to test compound/maximum response to isoprenaline. †,†Relative potency ratio compared with isoprenaline (isoprenaline = 1).

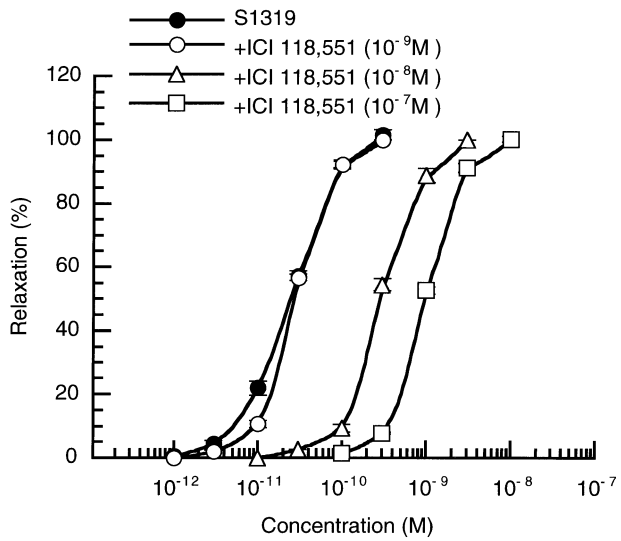

Figure 3 Concentration-response curves for tracheal relaxation by S1319 in the absence and the presence of ICI 118,551 obtained in tracheal preparations isolated from guinea-pigs. Precontraction was induced by 1×10^{-5} M histamine. Each point represents the mean of six preparations. Vertical bars represent the s.e.mean.

Concentration-response curves of S1319 for atrial chronotropism and inotropism were measured in guinea-pig isolated tissues (Table 1; data are presented as the mean±s.e.mean). The pD_2 value of S1319 for left atrial inotropism was less than that of isoprenaline and formoterol, and was equal to that of salbutamol. S1319 also produced a monophasic response for right atrial inotropism and was less potent than isoprenaline and formoterol. On the other hand, the pD_2 value of S1319 in the positive chronotropic response was greater than that of the other three agonists, being 603, 537 and 8.7 times the value of isoprenaline, salbutamol and formoterol, respectively. Isoprenaline produced a greater inotropic response than positive chronotropic response, whereas the stimulating effect of S1319 was greater on the positive chronotropic response than the inotropic response. The intrinsic activities of S1319 for the inotropic response of right and left atria were very low, being about half that of isoprenaline and formoterol.

Selectivity for β-adrenoceptors of tracheal smooth muscle vs those of cardiac muscle

Using data from isolated tracheal and atrial preparations of guinea-pigs, the selectivity of S1319, isoprenaline, salbutamol and formoterol for β-adrenoceptors was calculated from the pD_2 values (Table 2). The selectivity values of S1319 for the inotropic response of trachea (β_2) vs that of right and left atria (β_1) were 594±14 ($n=4$) and 8523±2454 ($n=6$), respectively, and were greater than for the other agonists. This ratio represents the usual selectivity, whereas organ-specific β_2-selectivity for the inotropic response of trachea (β_2) vs the chronotropic response of right atria (β_2) could be regarded as a measure of the therapeutic width (Voss *et al*., 1994). The latter ratio of S1319 was approximately 1.6, not much less than that of formoterol and more than that of isoprenaline and salbutamol (Table 2).

Duration of action of S1319 on contraction in the guinea-pig trachea

The off set of action was examined by removing the drug from the medium by a single change of bathing medium after submaximal relaxation induced by β-agonists. After washing to remove the β-agonists, methacholine was still present, so a decline of relaxation to the level of precontraction was observed (Figure 4). The maximal contraction of isoprenaline was reached within 30 min. For salbutamol the time to reach

Table 2 Selectivities of S1319, formoterol, salbutamol and isoprenaline for β-adrenoceptors in isolated tracheal and atrial preparations of guinea-pig

	*Selectivity ratio**		
Agonists	*Trachea/left atrium inotropism*	*Trachea/right atrium inotropism*	*Trachea/right atria chronotropism*
S1319	8523±2454	594±14	1.59±0.8
	(18743)†	(2181)	(1.9)
Formoterol	284±31	242±22	9.80±0.6
	(625)	(889)	(12)
Salbutamol	4.8±0.4	3.1±0.1	0.57±0.02
	(11)	(11)	(0.7)
Isoprenaline	0.45±0.02	0.27±0.01	0.81±0.07
	(1)	(1)	(1)

Values are given as the mean±s.e.mean. ($n=4-6$) *Selectivity = reciprocal of the ratio of D_2 in the respective tissues. †Relative potency ratio compared with isoprenaline (isoprenaline = 1).

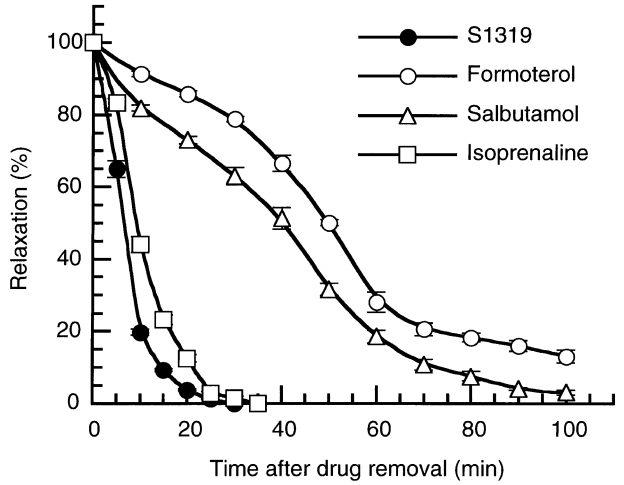

Figure 4 The effect in time of S1319 (1×10^{-8} M), isoprenaline (3×10^{-7} M), salbutamol (3×10^{-7} M) and formoterol (1×10^{-8} M) on the guinea-pig tracheal relaxation following removal of the agonists from the medium. Precontraction was induced by 1×10^{-5} M methacholine. Each point represents the mean of four preparations. Vertical bars represent the s.e.mean.

maximal contraction was longer and the maximal contraction of formoterol was not reached in the time-frame of the experiment (100 min). For S1319, maximal contraction was reached within 20 min. The estimated half-life of S1319, isoprenaline, salbutamol and formoterol was 4, 7, 37 and 48 min, respectively.

Discussion

The β-adrenoceptor stimulant activity of S1319 was examined using the isolated trachea and atria obtained from guinea-pigs. For this purpose, the *in vitro* pharmacology of S1319 has been compared with that of the reference compounds isoprenaline, which was reported to be a non-selective β-stimulant (Brittain *et al*., 1973), salbutamol (O'Donnell, 1972; Cullum *et al*., 1969) regarded as a selective agent, and formoterol (Ida, 1976; Nix *et al*., 1990) reported to have high selectivity and a long duration of action.

S1319 ($pD_2 = 10.58$) was approximately 1000 times more potent than isoprenaline ($pD_2 = 7.60$), 1200 times more potent than salbutamol ($pD_2 = 7.5$) and as potent as formoterol ($pD_2 = 10.52$) in relaxing tracheal smooth muscle obtained from guinea-pigs (Table 1). The concentration-response curve for S1319 was in parallel with those for isoprenaline, salbutamol and formoterol; it was shifted in a parallel fashion to the right in the presence of β_2-adrenoceptor selective antagonist ICI 118,551 (Figure 3). Furthermore, a radioligand binding experiment using the cell membrane expressing human β_1- or β_2-adrenoceptor demonstrated that S1319 possessed affinity and selectivity for β_2-adrenoceptor (Suzuki *et al*., 1999). These results suggest that S1319 is a β_2-adrenoceptor selective agonist that exerts a potent tracheal relaxing effect.

The intrinsic activity of S1319 in this tissue was close to 1.0, indicating that S1319 behaved as a β-adrenoceptor full agonist like the other three agonists. The dissociation constant for β_2-adrenoceptor of S1319 ($K_d = 51$ nM) (Suzuki *et al*., 1999) was approximately equal to that of isoprenaline ($K_d = 56$ nM) and salbutamol ($K_d = 110$ nM) (data from Voss *et al*., 1992). The efficacy of receptor interaction, calculated using the following equation (Haenen *et al*., 1990): $e = (D_2 + K_d)/D_2$ of S1319 is higher ($e = 1940$) than that of isoprenaline ($e = 3.2$) and salbutamol ($e = 4.5$). These results suggested that S1319 is a much more potent bronchodilating agent than isoprenaline and salbutamol.

The responses of S1319 in the guinea-pig heart can be used to obtain two selectivity ratios (Table 2): β_2/β_1 selectivity (trachea (β_2)/left or right atria inotropism (β_1)) and organ specific β_2 selectivity (trachea (β_2)/right atrial chronotropism (β_2)). The first ratio represents the usual selectivity, whereas the second could be regarded as a measure of therapeutic width (Voss *et al*., 1994). In this regard, S1319 is more active against β_2-adrenoceptors in tracheal smooth muscle than β_1-adrenoceptors in cardiac muscle and is found to be the most selective β_2-adrenoceptor agonist among the four tested agonists. The selectivity ratio (trachea/left atrial inotropism) of S1319 was 18743, 1776 and 30 times greater than those of isoprenaline, salbutamol and formoterol, respectively. Furthermore, the intrinsic activity of S1319 for the inotropism for the left and right atria (Table 1) indicate that S1319 is a β_1-adrenoceptor partial agonist. Since S1319 produced almost the same maximum tracheal relaxation as did the three other agonists (Figure 2), S1319 was much more active against tracheal smooth muscle than cardiac muscle. This inactivity for cardiac muscle of S1319 may contribute to its lack of cardiovascular effects when used as a bronchodilator.

Because in humans β_2-adrenoceptors are partly responsible for the chronotropic effects of β-adrenoceptor agonists (Brodde, 1991), the guinea-pig right atrium might be suitable as an *in vitro* model for the cardiac side effects of bronchodilators. The selectivity ratio (trachea/right atrial chronotropism) of S1319 was 1.9 and 2.8 times greater than those of isoprenaline and salbutamol, respectively. It was slightly less than that of formoterol. However, the preferable route of administration of β_2-adrenoceptor agonists in asthmatics is inhalation. In this way, the systemic concentration will be lower than the concentration in the airways and the lung, which provides a large therapeutic width.

As for the β_2-selectivity, that of S1319 was relatively high in the functional compared to the binding experiments where S1319 exhibited K_d values of 120 nM and 51 nM for β_1- and β_2-adrenoceptor membrane preparations from CHO cells stably expressing the adrenoceptors (Suzuki *et al*., 1999). Although the reason for this discrepancy is not clear, the antagonistic activities of S1319 may have increased the selectivity in the functional experiments. The antagonistic activity of S1319 in the isolated left atria is considered to be greater than that of formoterol, salbutamol, and isoprenaline, because S1319 has less intrinsic activity than these agonists. This antagonistic effect of S1319 was not reflected in the binding experiment. Secondly, the lower efficacy of the β_1-adrenoceptor interaction may have increased the selectivity. The dissociation constant for β_1-adrenoceptor of S1319 ($K_d = 120$ nM) (Suzuki *et al*., 1999) was smaller than that of isoprenaline ($K_d = 951$ nM) and salbutamol ($K_d = 22800$ nM) (data from Kikkawa *et al*., 1997). When the efficacy of β_1-adrenoceptor interaction was calculated as described above, the efficacy of S1319 ($e = 2.1$) was found to be approximately equal to that of isoprenaline ($e = 1.8$) and salbutamol ($e = 1.7$). Since the β_2-adrenoceptor efficacy ($e = 1940$) is much higher than the β_1-adrenoceptor efficacy ($e = 2.1$), S1319 needs less β_2- than β_1-adrenoceptors to be occupied for maximal effect. What is clear, however, is that S1319 appears to possess a high degree of selectivity for β_2-adrenoceptors. Potency is a function of both receptor affinity and density, and efficacy of G protein coupling. Ultimately, further experiments on other animal species *in vitro* and *in vivo* will be needed to draw a firm conclusion on the

selectivity of S1319, because the β_2/β_1 selectivity of agonists differs according to the animal species and experimental conditions used (Bowman & Raper, 1976).

The duration of the action is an important feature of β_2-adrenoceptor agonists. Experiments were performed in which the drug was removed from the medium by washing, after inducing submaximal relaxation. In the isolated trachea preparation, metabolism cannot play a substantial role. Therefore, the results have to be explained by drug-receptor interaction. After removing S1319 from the organ bath by washing in the presence of methacholine, an almost instantaneous return to the precontraction level was observed. However, in similar experiments with salbutamol and formoterol, the return to the precontraction level was slower and not complete within the duration of the experiment with formoterol (Figure 4). Consequently, it would appear that S1319 bound to the receptor is responsible for the shortening of the duration of action as compared to salbutamol and formoterol. It is suggested that the mechanism by which salmeterol, a long-acting β_2-adrenoceptor agonist, exhibits its action involves binding to a specific exo-site in the cell membrane adjacent to the β-adrenoceptor (Bradshaw *et al*., 1987), and may result in part from the lipid solubility of the compound (Nials *et al*., 1993). It is suggested that for β_2-adrenoceptor agonists, long duration of action may depend upon several factors. Both formoterol and salmeterol display a higher lipophilicity and have a higher affinity, selectivity, and potency than most short-acting agonists at the β_2-adrenoceptor. Of the factors, lipophilicity may prove to be the most important one by determining the amount of the drug entering into the cell membrane in the vicinity of the β_2-adrenoceptor (Linden *et al*., 1996; Waldeck, 1996). Indeed, the rank order of offset action for the four agonists included in this study corresponded with the rank order of the lipophilicities of the molecules.

In conclusion, S1319, the first bronchodilator derived from marine sponge, is a potent and selective β_2-adrenoceptor agonist with a short duration of action in relaxing airway smooth muscle *in vitro*. We present the first evidence that a sympathomimetic amine with a benzothiazolone ring has the potency of efficacious β_2-adrenoceptor agonists. S1319 and its derivatives are suggested to be potential bronchodilating agents useful for the treatment of patients with reversible airway obstruction.

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