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Effects of L-serine borate on antagonism of leukotriene C_4-induced contractions of guinea-pig trachea

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1 The antagonist activity of three leukotriene D_4 (LTD_4) receptor antagonists and a number of bronchodilators was determined against LTC_4-induced contractions of guinea-pig isolated tracheal chains in the absence and presence of the γ-glutamyltranspeptidase inhibitor, L-serine borate (SB).

2 The LTD_4 receptor antagonists FPL-55712, L-649,923 and L-648,051 effectively antagonized LTC_4 responses in the absence of SB but were ineffective in the presence of 15 and/or 45 mM SB.

3 Salbutamol > isobutylmethylxanthine (IBMX) > dibutyryl cyclic AMP > aminophylline > nifedipine antagonized contractions to LTC_4 in the absence of SB. In contrast, in the presence of SB the antagonist activity of all of these agents except nifedipine was significantly reduced. The antagonist activity of the Ca^{2+} entry blocker, nifedipine, was similar in the absence and presence of SB.

4 Salbutamol and IBMX were potent functional antagonists of LTE_4-induced contractions both in the absence and presence of SB.

5 These results are consistent with the hypothesis that there are contractile LTC_4 receptor mechanisms in guinea-pig trachea which are unmasked by SB and are not blocked by LTD_4 receptor antagonists and which are less effectively down modulated by cyclic AMP-dependent bronchodilators.

Introduction

Pharmacological experiments (Drazen *et al* 1980; Krell *et al.*, 1981; 1983; Fleisch *et al.*, 1982; 1985; Jones *et al.*, 1983; 1986a, b; Snyder & Krell, 1984) and radioligand binding studies (Pong & DeHaven, 1983; Pong *et al.*, 1983; Mong *et al.*, 1984; 1985; Cheng & Townley, 1984; Lewis *et al.*, 1984; Rovatti *et al.*, 1985) support the existence of sulphidopeptide leukotriene receptor subtypes in lung from a number of species. Specifically, separate leukotriene C_4 (LTC_4) and LTD_4 binding sites have been identified and characterized (Mong *et al.*, 1984; 1985; Cheng & Townley, 1984). Functional studies on guinea-pig airway smooth muscle suggest that LTC_4 and LTD_4 can elicit contraction through an interaction with LTD_4 receptors (Krell *et al.*, 1981; Jones *et al.*, 1983; 1986a, b; Snyder & Krell, 1984; Buckner *et al.*, 1986). However, in the presence of the γ-glutamyltranspeptidase inhibitor, L-serine borate (SB), LTC_4 appears to produce contraction through a separate and distinct receptor which, at least in guinea-pig trachea (Snyder & Krell; 1984; Buckner *et al.*, 1986), is insensitive to the blocking actions of the LTD_4 antagonist, FPL-55712. Although current findings in leukotriene research are consistent with the hypothesis that LTD_4 receptors are the most pharmacologically important in terms of airway smooth muscle contraction (Mong *et al.*, 1985; Buckner *et al.*, 1986), it is still important to characterize LTC_4 and LTD_4 receptors and mechanisms. In the present paper we present results from functional studies on guinea-pig trachea which assessed the effects of selective LTD_4 receptor antagonists, the Ca^{2+}-entry blocker nifedipine and a number of bronchodilators on contractions initiated through these two putative receptor types. This was achieved by comparing pharmacological interactions of these agents with LTC_4 both in the absence and presence of the γ-glutamyltranspeptidase inhibitor SB.

Methods

Tracheal chains from male Hartley guinea-pigs (300–500 g) were prepared as previously described (Jones *et al.*, 1983; 1986a, b). Tissues were suspended in 20 ml organ baths containing 10 ml of a modified Krebs buffer at 37 ± 0.5°C and gassed continuously with 95% O_2 and 5% CO_2.

The composition of this Krebs bicarbonate buffer was (mM) KCl 4.72, $CaCl_2$ 2.5, $MgSO_4 . 7H_2O$ 0.6, KH_2PO_4 1.2, NaCl 120, dextrose 11, $NaHCO_3$ 25. The

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pH of the bathing solution was 7.4 ± 0.05. The cyclooxygenase inhibitor, indomethacin (1.4 μM), and the muscarinic receptor antagonist, atropine (0.1 μM) were added to the buffer to block spontaneous tone development and the effects of any spontaneously released acetylcholine, respectively. Contractions were recorded isometrically using Grass Force displacement transducers (Model FT03, Grass Instruments Co., Quincy, Mass.) connected by strain gauge couplers to a Beckman Type R Dynograph.

Tissues were placed under 1 g tension and allowed to equilibrate for 20 to 30 min, during which period they were automatically washed every 3 min (20 ml volume replacement). They were primed 2–3 times with histamine (90 μM) and once with a submaximal concentration of the agonist under study. Primings were separated by washing cycles that allowed recovery of baseline tensions.

A single cumulative dose-response curve to LTC_4 or LTE_4 was subsequently obtained on each tissue in the absence (control) or in the presence of 15 or 45 mM SB (30 min pretreatment). All tissues were pretreated with 0.7 μM mepyramine for 20 min before starting the cumulative dose-response curve. This procedure (of adding mepyramine) served to reduce variable spontaneous contractions which were sometimes encountered when carrying out this type of experiment.

The effects of the LTD_4 receptor antagonists, L-649,923, L-648,051 and FPL-55712, the Ca^{2+} entry blocker, nifedipine, and a number of bronchodilators, were determined against the responses to LTC_4 in the absence and presence of SB. Additional experiments were included to determine the effects of some of the above treatments on the dose-response curve to LTE_4 in the absence and presence of SB.

Analyses

In order to normalize the data, all responses were expressed as a percentage of the maximum contraction to histamine, which was determined on each tissue before constructing the dose-response curve. Individual EC_{50} values (concentration producing 50% of the maximum response to histamine) for control and treatment curves were calculated using linear regression analysis applied to the linear portion of each dose-response curve. The effects of the various treatments both in the absence and presence of SB were determined by calculating dose-ratios in each individual experiment. The dose-ratios were calculated according to the following equation: EC_{50} test/EC_{50} control.

Differences between geometric dose-ratios in the absence, as compared to the presence, of SB were considered significant at $P < 0.05$ as determined by a Student's t test for non-paired data or by a Mann-Whitney test when it was required (non-parametric).

Materials

Synthetic leukotrienes C_4 and E_4, L-649,923 (sodium (βS*, γR*)-4-(3-(4-acetyl-3-hydroxy-2-propylphenoxy) propylthio) - γ - hydroxy - β-methylbenzenebutanoate), L-648,051 (sodium 4-[3-(4-acetyl-3-hydroxy-2-propylphenoxy) propylsulphonyl] - γ - oxobenzenebutanoate), FPL-55712 (dissolved in twice distilled H_2O), indomethacin (dissolved in 100% ethanol) were synthesized in the Medicinal Chemistry Department, Merck Frosst Canada Inc. Histamine diphosphate, atropine sulphate, mepyramine maleate, salbutamol, nifedipine dissolved in DMSO, 3-isobutyl-1-methylxanthine (IBMX) dissolved in DMSO; N^6-2′-O-adenosine 3′:5′-cyclic monophosphate (dibutyryl cyclic AMP) dissolved in DMSO; and aminophylline dissolved in twice distilled H_2O were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). All drugs were dissolved in isotonic saline (0.9% NaCl w/v) unless indicated otherwise.

L-Serine borate (SB) was prepared by combining equimolar (2 M) concentrations of L-serine and boric acid dissolved in water and buffered at pH 7.4 with small amounts of 10 M NaOH. Final stock concentration was 1 M and 150 μl or 450 μl of that solution was added to give 15 or 45 mM final bath concentrations.

Results

Effects of serine borate on responses to leukotriene C_4

LTC_4-induced contractions were consistently potentiated by the presence of SB. This resulted in a concentration-dependent leftward shift of the dose-response curve for LTC_4. On average, 15 mM and 45 mM SB produced an approximate 2.5 and 9 fold decrease in the EC_{50} for LTC_4 compared to control, respectively. The control EC_{50} was 9.3 ± 1.9 nM ($n = 10$) compared to 4.0 ± .7 nM ($n = 6$) and 1.0 ± 0.3 nM ($n = 4$) in the presence of 15 mM and 45 mM SB, respectively.

Effects of the leukotriene D_4 receptor antagonists on responses to leukotriene C_4 in the presence or absence of L-serine borate

The effects of SB on the blocking activity of three LTD_4 receptor antagonists are summarized in Table 1. In the absence of SB the three antagonists L-649,923 L-648,051 and FPL-55712 all produced concentration-dependent parallel rightward shifts in the LTC_4 dose-response curve. The antagonist action is indicated by the dose-ratios which represent a ratio of the EC_{50} values obtained in presence and absence of antagonist (Table 1). In the presence of 2 μM FPL-55712, L-648,051 or L-649,923, the EC_{50} values for the

Table 1 Effects of leukotriene D_4-receptor antagonists on leukotriene C_4-induced contractions of guinea-pig trachea

Antagonist	(μM)	*Dose-ratio** (*no serine borate*)	*Dose-ratio* (15 mM serine borate)
FPL-55712	2	8.16 ± 1.9 ($n = 3$)	2.30 ± 1.28 ($n = 3$)
	6	13.16 ± 1.3 ($n = 4$)	1.88 ($n = 1$)
L-649,923	2	10.1 ± 1.5 ($n = 3$)	2.12 ± 1.3** ($n = 3$)
	6	21.1 ± 1.8 ($n = 3$)	2.97 ± 1.4** ($n = 3$)
	20	37.6 ± 1.5 ($n = 3$)	3.85 ± 1.0** ($n = 3$)
L-648,051	2	10.1 ± 1.0 ($n = 2$)	2.15 ± 1.6 ($n = 3$)
	6	N.D.	1.42 ± 1.6 ($n = 3$)
	20	N.D.	2.16 ± 1.4 ($n = 3$)

*Dose-ratio represents mean dose-ratio ± s.e.mean obtained from EC_{50} test/EC_{50} control in each individual (n) experiment.
**Significantly different from control ratio at $P < 0.05$ level.

LTC_4 were increased 8, 10 and 10 fold, respectively. In the presence of 6 μM FPL-55712 and L-649,923, the EC_{50} values for LTC_4 were increased 13 and 21 fold, respectively. A limited number of studies were carried out with 20 μM L-649,923 and in the presence of this concentration of antagonist, the EC_{50} for LTC_4 was increased approximately 38 fold.

In contrast, the effectiveness of these three drugs as leukotriene C_4 antagonists was markedly reduced on tissues treated with 15 mM SB (compare dose-ratios in absence and presence of SB in Table 1). This is particularly evident with L-649,923 and L-648,051 (2–20 μM) which now produced only 2–4 fold shifts in the LTC_4 dose-response curves. Some additional studies were carried out in the presence of 45 mM SB. In the presence of this concentration of SB, 6 μM FPL-55712 ($n = 4$) was essentially inactive as an antagonist, producing only a 2 fold increase in the EC_{50} to LTC_4. These results are identical to those obtained with this antagonist in the presence of 15 mM SB (Table 1).

Effects of nifedipine on responses to leukotriene C_4 in the presence or absence of L-serine borate

The Ca^{2+}-entry blocker nifedipine was tested using concentrations previously shown to produce partial blockade of the responses to LTC_4 on guinea-pig trachea (Jones *et al.*, 1984). Nifedipine displayed similar antagonist activity versus LTC_4 both in the absence and presence of 15 mM SB. Nifedipine (0.3 μM) produced a 5.5 ± 1.6 fold ($n = 7$) and a 7.3 ± 2.2 fold ($n = 3$) shift to the right of the LTC_4 dose-response curve in the absence and presence of 15 mM SB, respectively. Similar shifts to the right were obtained in 3 experiments in the presence of 45 mM SB. In these experiments, nifedipine depressed the maximum response to LTC_4 from 94% to 90% in the absence of SB, and from 106% to 87% in the presence of 45 mM SB. Increasing the concentration of nifedipine to 3 μM ($n = 3$) or 30 μM ($n = 3$) did not produce any further shifts in the LTC_4 dose-response curve or depressions of the maximum responses either in the absence or presence of SB.

Effects of phosphodiesterase inhibitors on responses to leukotriene C_4 in the presence or absence of L-serine borate

The phosphodiesterase inhibitor aminophylline was a relatively weak functional antagonist of LTC_4-induced contractions at the concentrations tested. This methylxanthine produced only minimal rightward shifts in the dose-response curve to LTC_4 in the absence of SB. In the presence of 48–190 μM ($n = 9$) aminophylline, the dose-response curve to LTC_4 was shifted approximately 4–12 fold. No significant alterations in the maximum responses to LTC_4 were observed. However, in the presence of 15 mM SB, 190 μM aminophylline ($n = 3$) was essentially inactive, producing only an approximate 1.6 fold shift in the dose-response curve to LTC_4. Additional studies were carried out with a more potent phosphodiesterase

Table 2 Effect of serine borate on the functional antagonism of leukotriene C_4-induced contractions of guinea-pig trachea induced by various bronchodilators

		Dose-ratios*		
		Control (no serine borate)	*Serine borate*	
Drug	*Conc. (μM)*		*15 mM*	*45 mM*
IBMX	30	97.1 ± 1.6 ($n = 9$)	4.3 ± 1.6** ($n = 4$)	11.8 ± 1.2** ($n = 5$)
Dibutyryl cyclic AMP	300	154.8 ± 1.9 ($n = 4$)	N.D.	2.8 ± 1.1** ($n = 4$)
Salbutamol	0.13	31.0 ± 1.4 ($n = 10$)	4.7 ± 1.3** ($n = 6$)	1.0 ± 1.2** ($n = 4$)
	0.42	86.1 ± 1.5 ($n = 4$)	8.5 ± 1.5** ($n = 4$)	N.D.

*Mean ± s.e.mean dose-ratio determined as described in Table 1.
**Significantly different from control (i.e. no serine borate present) at $P < 0.05$ level. IBMX = isobutylmethylxanthine.

inhibitor, isobutylmethylxanthine (IBMX). This agent was a much more potent antagonist of LTC_4-induced contractions, producing on average a 97.0 fold shift in the dose-response curve at 30 μM (Table 2). In contrast to the results obtained with the leukotriene receptor antagonists, this agent produced non-parallel shifts in the dose-response curve to LTC_4 (Figure 1a). This was particularly evident with higher concentrations of IBMX (100 μM) where the maximum response to LTC_4 was reduced to 35 ± 2.5% of control ($n = 3$). However, in the presence of SB, the antagonist activity of IBMX was significantly attenuated. At 30 μM IBMX now produced only a 4 and 12 fold shift in the dose-response curve to LTC_4 (Figure 1b and Table 2) in the presence of 15 mM and 45 mM SB, respectively.

Effects of dibutyryl cyclic AMP on responses to leukotriene C_4 in the presence or absence of L-serine borate

The results obtained with another bronchodilator, dibutyryl cyclic AMP (300 μM) are displayed in Figure 2 and summarized in Table 2. Similar to IBMX, this cyclic AMP mimic produced a non-parallel, rightward shift in the dose-response curve to LTC_4 in the absence of SB (dose-ratio 154.8 ± 1.9 at the EC_{50} level, Table 2). In the presence of 45 mM SB, this cyclic AMP analogue was significantly less effective at preventing the contractions to LTC_4. Under these conditions, dibutyryl cyclic AMP was essentially inactive, producing only a 2.8 ± 1.1 fold shift in the dose-responce curve to LTC_4.

Effects of salbutamol on responses to leukotriene C_4 in the presence or abscence of L-serine borate

The β_2-adrenoceptor agonist salbutamol was a very effective functional antagonist of LTC_4-induced contractions. This agent produced a concentration-dependent rightward shift in the dose-response curve to LTC_4 (Table 2). In the presence of 15 mM SB the antagonist activity of 0.13 μM and 0.42 μM salbutamol was substantially reduced (Table 2). This reduction in functional antagonist activity was even greater when 0.13 μM salbutamol was tested in the presence of 45 mM SB (Figure 3 and Table 2). Under these experimental conditions 0.13 μM salbutamol failed to alter significantly the dose-response curve to LTC_4 (dose-ratio 1.0 ± 1.2 versus 31.0 ± 1.4 obtained on control tissues, Table 2).

Studies with leukotriene E_4 in the presence or abscence of L-serine borate

It is generally assumed that in the absence of SB, LTC_4 produces contractions through an interaction with LTD_4 receptors as a result of metabolism to LTD_4 (Snyder & Krell, 1984). In order to test the specificity of the interaction of SB with LTC_4, we carried out a limited number of experiments with LTE_4. This leukotriene is not metabolized by γ-glutamyltranspeptidase and is thought to produce its effects through an interaction with the LTD_4 receptor (Pong & DeHaven, 1983; Krell *et al.*, 1983; Jones *et al.*, 1986a, b). IBMX

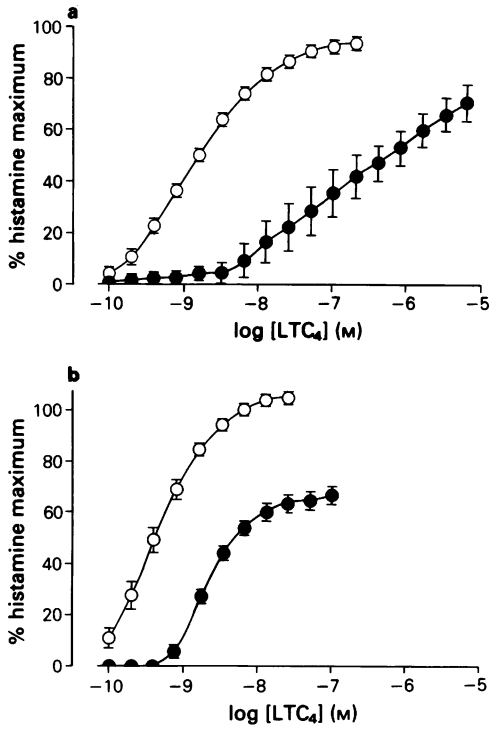

Figure 1 Effect of 30 μM isobutylmethylxanthine (IBMX ●; $n = 5$ in (a) and (b)) on the log concentration-response curve for contraction to leukotriene C_4 (LTC_4, ○; $n = 5$ in (a) and (b)) of guinea-pig trachea in the absence (a) and presence (b) of 45 mM L-serine borate complex. Symbols represent mean responses expressed as a % of the maximum histamine contraction; vertical lines show s.e.mean. All studies were carried out in the presence of 1.4 μM indomethacin, 0.1 μM atropine and 0.7 μM mepyramine. Note that IBMX was a significantly more effective functional antagonist in the absence of serine borate (a) than in its presence (b) ($P < 0.05$).

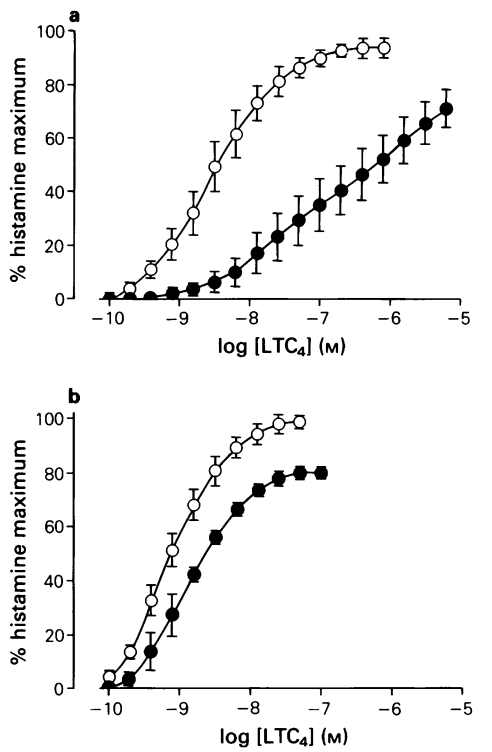

Figure 2 Effect of 300 μM dibutyryl cyclic AMP (●; $n = 4$ in (a) and (b)) on the log concentration-response curve for contraction to leukotriene C_4 (LTC_4, ○; $n = 4$ in (a) and (b)) of guinea-pig trachea in the absence (a) and presence (b) of 45 mM L-serine borate complex. Symbols represent mean responses expressed as a % of the maximum histamine contraction; vertical lines show s.e.mean. All studies were carried out in the presence of 0.14; μM indomethacin, 0.1 μM atropine and 0.7 μM mepyramine. Note that dibutyryl cyclic AMP was a significantly more effective functional antagonist in the absence of serine borate (a) than in its presence (b) ($P < 0.01$).

(Figure 4) and salbutamol (Figure 5) were very potent antagonists of LTE_4-induced contractions. The interaction of these bronchodilators, however, was quantitatively and qualitatively different from that observed with LTC_4. Both compounds produced rightward shifts in the dose-response curves to LTE_4 but also dramatically reduced the maximal contraction to this leukotriene both in the absence and presence of SB (45 mM). The greater sensitivity of LTE_4 to functional antagonism was indicated by the fact that 3 fold lower concentrations of IBMX and salbutamol were effective. IBMX depressed maximum responses to LTE_4 from 89 ± 3% to 58 ± 4% ($P < 0.001$) in control and from 77 ± 4% to 47 ± 4% ($P < 0.001$) in the presence of 45 mM SB. Salbutamol reduced the maximum contractions to LTE_4 from 84 ± 2% to 45 ± 4% ($P < 0.01$) in control and from 66 ± 2% to 26 ± 8% ($P < 0.01$) in the presence of 45 mM SB. Due to the reduction in the maximum contraction to LTE_4 induced by these bronchodilators, dose-ratios could be determined for IBMX at the EC_{35} level only. Similar dose-ratios were obtained in the control (7.2 ± 1.5) and SB-treated tissues (7.6 ± 2.7 fold). Thus, in contrast to the results obtained with LTC_4, these bronchodilators retained their antagonist activity against LTE_4 in the presence of 45 mM SB.

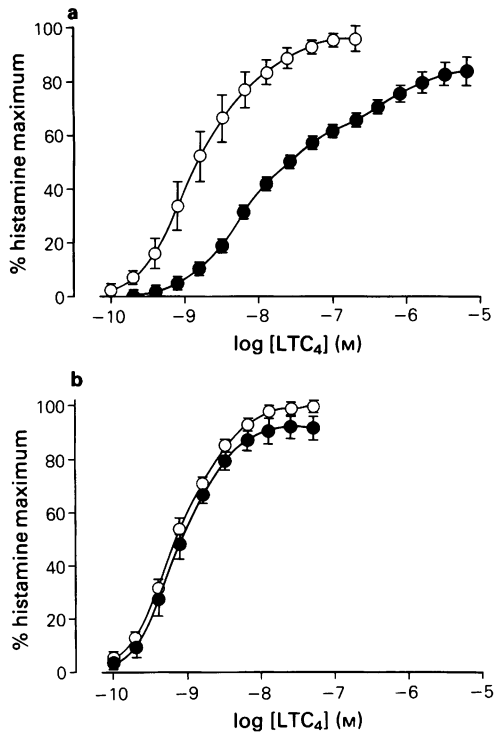

Figure 3 Effect of 0.13 μM salbutamol (●; $n = 4$ in (a) and (b)) on the log concentration-response curve for contraction to leukotriene C_4 (LTC_4, ○; $n = 3$ in (a) and (b)) of guinea-pig trachea in the absence (a) and presence (b) of 45 mM L-serine borate complex. Symbols represent mean responses expressed as a % of the maximum histamine contraction; vertical lines show s.e.mean. All studies were carried out in the presence of 0.14 μM indomethacin, 0.1 μM atropine and 0.7 μM mepyramine. Note that salbutamol was a significantly more effective functional antagonist in the absence of serine borate (a) than in its presence (b) ($P < 0.05$).

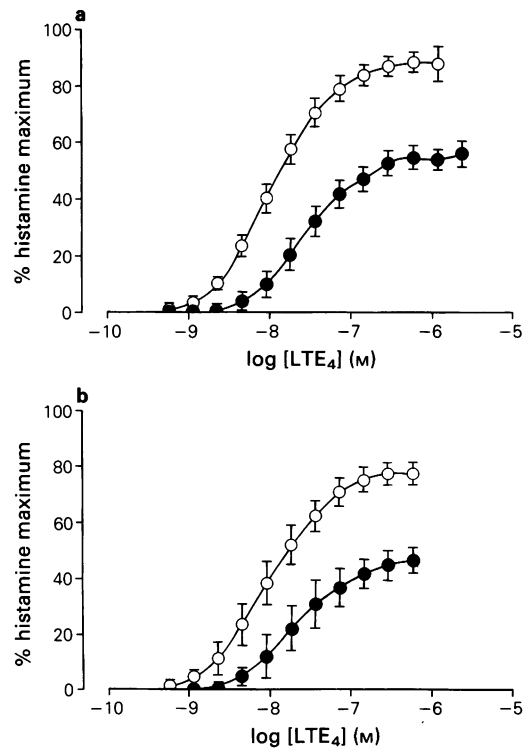

Figure 4 Effect of 10 μM isobutylmethylxanthine (IBMX, ●; $n = 6$ in (a) and $n = 3$ in (b)) on the control log concentration-response curve for contraction to leukotriene E_4 (LTE_4, ○, $n = 6$ in (a) and $n = 3$ in (b)) of guinea-pig trachea in the absence (a) and presence (b) of 45 mM L-serine borate complex. Symbols represent mean responses expressed as a % of the maximum histamine contraction; vertical lines show s.e.mean. All studies were carried out in the presence of 0.14 μM indomethacin, 0.1 μM atropine and 0.7 μM mepyramine. Note that there was no significant difference in the functional antagonist activity of IBMX in the absence (a) and presence (b) of serine borate ($P < 0.05$).

Discussion

The present findings confirm and extend previous results that SB selectively potentiates contractile responses to LTC_4 on guinea-pig trachea and renders these responses less susceptible to LTD_4 receptor blockers (Snyder & Krell, 1984). In the presence of SB, LTD_4 antagonists, such as FPL-55712 (Snyder & Krell, 1984) and two recently described LTD_4 receptor antagonists, L-649,923 (Jones *et al.*, 1986b) and L-648,051 (Jones *et al.*, 1986a) were significantly less effective at blocking LTC_4-induced contractions. These findings support the hypothesis that if metabolism of LTC_4 to LTD_4 is inhibited, then a contraction to LTC_4 can be produced through a receptor mechanism which is pharmacologically different from the LTD_4 receptor (Snyder & Krell, 1984). Based on these observations, we and others (Snyder & Krell, 1984) have assumed that responses of guinea-pig trachea induced by LTC_4 in the absence and presence of SB represent activation of LTD_4 and LTC_4 receptors, respectively. Metabolic (Snyder *et al.*, 1984; Aharony *et al.*, 1985) and specific receptor binding studies (Hogaboom *et al.*, 1983; Cheng & Townley, 1984; Mong *et al.*, 1984) with LTC_4, LTD_4 and/or LTE_4 in the absence and presence of SB also support this

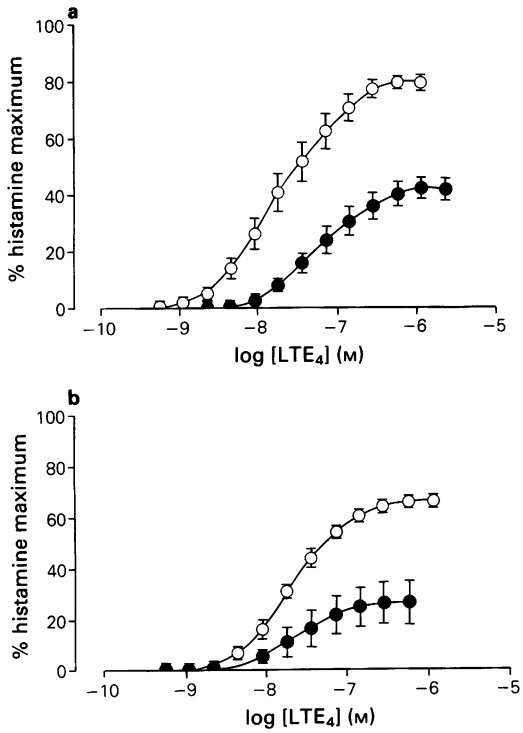

Figure 5 Effect of 0.042 μM salbutamol (●; $n = 11$ in (a) and $n = 5$ in (b)) on the log concentration-response curve for contraction to leukotriene E_4 (LTE_4, ○; $n = 11$ in (a) and $n = 5$ in (b)) of guinea-pig trachea in the absence (a) and presence (b) of 45 mM L-serine borate complex. Symbols represent mean responses expressed as a % of the maximum histamine contraction; vertical lines show s.e.mean. All studies were carried out in the presence of 0.14 μM indomethacin, 0.1 μM atropine and 0.7 μM mepyramine. Note that there was no difference in the functional antagonist activity of salbutamol in the absence (a) and presence (b) of serine borate.

hypothesis. Although several critical criteria for defining a receptor (e.g. potent and specific LTC_4 receptor antagonist) have not yet been met for LTC_4 in guinea-pig trachea, this tissue still provides a unique and useful functional model which can be employed to investigate LTC_4-induced responses and interactions.

A major goal of this study was to determine the influence of SB on the degree of functional antagonism of LTC_4 produced by different types of known bronchodilators. The present findings obtained with nifedipine in the absence and presence of SB are consistent with previous observations that Ca^{2+} entry blockers produce only a modest rightward shift in the dose-response curves to leukotrienes (Jones *et al.*, 1984; Jones & Denis, 1985). However, these results do not allow one to determine whether the nifedipine-insensitive responses observed in 2.5 mM Ca^{2+} to LTC_4 and LTD_4 receptor stimulation are mediated through different Ca^{2+} mobilizing pathways (Jones & Denis, 1985). Previously Weichman & Tucker (1985) concluded from studies with TMB-8 that these receptors once activated, mobilized Ca^{2+} in a qualitatively similar manner. Additional studies are required to support this hypothesis.

In contrast, the results obtained with the cyclic AMP-dependent bronchodilators were dramatically altered in the presence of SB. The efficacies of the β-adrenoceptor agonist salbutamol, the phosphodiesterase inhibitor IBMX and the cyclic AMP mimic dibutyryl cyclic AMP on responses to LTC_4 were markedly reduced, compared to control responses obtained in the absence of SB. These findings indicate that transduction mechanisms following LTC_4 receptor stimulation are different from those activated by LTD_4. This hypothesis receives support from the observations that functional antagonism of the LTD_4 receptor agonist, LTE_4, was not similarly affected by SB. Moreover, these findings illustrate that the results observed in the presence of SB were not simply due to an effect of SB on inhibitory mechanisms in general. It is impossible from the present studies to determine the precise nature or locus of these differences but a clue is provided from the observations that three different classes of cyclic AMP-dependent bronchodilators were affected in a qualitatively similar manner by SB. Although there were some minor quantitative differences between these agents the present findings suggest that the altered sensitivity to these relaxants is largely a reflection of events that operate after the generation of cyclic AMP. Additional studies with bronchodilators that act through other mechanisms are required to support this hypothesis.

β-Adrenoceptor agonists and phosphodiesterase inhibitors are thought to produce relaxation of airway smooth muscle by elevating levels of cyclic AMP which leads to an activation of a cyclic AMP-dependent protein kinase (Andersson *et al.*, 1980). The relaxant effect is postulated to be mediated by specific protein kinase catalysed phosphorylation reactions which could increase intracellular Ca^{2+} sequestration, increase Ca^{2+} extrusion and/or could decrease the activity of myosin light chain kinase (Andersson *et al.*, 1980; Daniel *et al.*, 1983). The net effect of decreasing intracellular Ca^{2+} is to decrease contractility. It follows that the activity of various bronchodilators will be dependent on the way a contractile receptor is coupled to the contractile apparatus. The potency of β-adrenoceptor agonists is known to be dependent on the degree of spontaneous tone of the airway preparation as well as the choice of spasmogen used to induce

tone (Van den Brink, 1973; Jones *et al.*, 1974; Buckner & Saini, 1975; Torphy *et al.*, 1983; Torphy, 1984). β-Adrenoceptor agonists are very effective at relaxing or preventing leukotriene-induced contractions in human (Jones *et al.*, 1982) and guinea-pig trachea (Armour *et al.*, 1982; Torphy *et al.*, 1983) but are less effective on responses to cholinoceptor agonists (Torphy *et al.*, 1983). The differences were thought to reflect differences in the pathways utilized for mobilization of Ca^{2+} although it has been found that some spasmogens, such as methacholine, can actually decrease the ability of isoprenaline to increase cyclic AMP accumulation (Torphy *et al.*, 1983). Torphy *et al.* (1986) investigated this phenomenon further by studying these interactions at a pharmacological and a biochemical level. They concluded that cholinoceptor stimuli decreased β-adrenoceptor agonist activity to a greater extent than did LTD_4. However, in contrast to previous studies in another species (Torphy *et al.*, 1985) this did not appear to be due to differences in the ability to generate cyclic AMP but to a difference in sensitivity to cyclic AMP. LTC_4 receptor-mediated responses display some similarities to cholinoceptor agents in this respect, although in the absence of SB this leukotriene was also found to lower cyclic AMP levels in guinea-pig trachea (Andersson *et al.*, 1982). A recent study has confirmed our findings with salbutamol and LTC_4 on guinea-pig trachea (Hay *et al.*, 1986). These researchers also observed that the altered sensitivity to salbutamol is greater if it is added before rather than after induction of contraction to LTC_4, which raises the possibility that mechanisms involved in initiation and maintenance of contraction are affected differently by β-adrenoceptor agonists.

The present findings have important implications in terms of respiratory diseases due to the fundamental nature of processes which function through a cyclic AMP second messenger. Thus, if excessive LTC_4 is produced within the lung, its effects could be even more detrimental than those of LTD_4, if airway smooth muscle contraction to it is less sensitive to functional antagonism by exogenous and endogenous bronchodilators. However, the significance of LTC_4 receptors in human airway smooth muscle contraction has been questioned because, in contrast to guinea-pig trachea, LTC_4-induced contractions of human intralobar bronchioles (Buckner *et al.*, 1986) in the presence of SB were blocked by LTD_4 receptor antagonists, such as FPL-55712. Thus, although specific LTC_4 binding sites (Rovati *et al.*, 1985) have been found to be present in human lung tissue, they may function only to modulate rather than mediate contractions. Furthermore, the importance of receptor subtypes in human lung pathophysiology could be dependent on the presence or absence of disease or regional differences in the distribution of LTC_4 and LTD_4 receptors.

In conclusion, the present findings confirm previous data that there are functionally relevant contractile LTC_4 receptors in guinea-pig trachealis. These responses are not blocked by LTD_4 receptor antagonists, are partially antagonized by nifedipine and display reduced sensitivity to functional antagonism by a variety of cyclic AMP-dependent bronchodilators. Parallel biochemical and pharmacological studies are required in order to study these interactions at a more fundamental level.

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