Br. J. Pharmac. (1986), **87**, 741–749

Aerosolized and intravenously administered leukotrienes: effects on the bronchoconstrictor potency of histamine in the guinea-pig

M.R. Fennessy, A.G. Stewart[1] & D.C. Thompson

Department of Pharmacology, University of Melbourne, Parkville, 3052, Australia

1 The effects of leukotrienes C_4 and D_4 (LTC_4 and LTD_4), administered intravenously or by aerosol, on the bronchoconstrictor potency of intravenously administered histamine have been investigated in anaesthetized, mechanically ventilated guinea-pigs.

2 LTC_4 (2 nM) had no effect on either the EC_{50} or the maximum contractile response to histamine on the isolated trachea of the guinea-pig. At 10 nM, LTC_4 induced a rightward shift in the histamine concentration-response curve without affecting the maximum response.

3 LTD_4 (0.05–0.20 nmol kg^{-1}, i.v.) dose-dependently enhanced histamine (9–36 nmol kg^{-1}, i.v.)-induced increases in airways resistance, whereas equibronchoconstrictor doses of LTC_4 (0.1–0.4 nmol kg^{-1}, i.v.), failed to enhance histamine-induced increases in airways resistance.

4 Aerosols of LTC_4 and LTD_4 generated from solutions of 1–16 μM and administered for 30 s, elicited concentration-dependent bronchoconstrictions comprising decreases in dynamic compliance and increases in airways resistance. At 20 min after exposure to these aerosols, the potency of histamine (9–36 nmol kg^{-1}, i.v.) was significantly increased on both airways resistance and dynamic compliance.

5 The potentiation induced by LTC_4 (4 μM, 30 s) was maintained up to 60 min after aerosol exposure whereas that induced by LTD_4 (4 μM, 30 s) was maintained up to 40 min after aerosol exposure but was not significantly different ($P > 0.05$, unpaired Student's t test) to saline-exposed animals at 60 min.

6 LTC_4, as has been previously reported for LTD_4, does not enhance the histamine-induced contraction of isolated airways smooth muscle. In contrast to LTD_4, intravenously administered LTC_4 does not appear to enhance histamine-induced bronchoconstriction. On the other hand, aerosols of either LTC_4 or LTD_4 potentiate histamine *in vivo* in a concentration-dependent manner. These data suggest that leukotrienes may contribute to the regulation of airways reactivity to histamine in the guinea-pig.

Introduction

The sulphidopeptide leukotrienes (LTC_4, LTD_4 and LTE_4) have been implicated as primary mediators of anti-histamine-resistant airways smooth muscle constriction induced by antigen-challenge (Adams & Lichtenstein, 1979). In addition, these substances together with the chemoattractant leukotriene, LTB_4, are considered to be important mediators in inflammatory conditions (Samuelsson, 1983). A number of studies suggest that airways inflammation may be responsible for the increased airways reactivity which is a characteristic feature of asthma (Empey *et al.*, 1976; Hogg, 1981; Cartier *et al.*, 1982; Malo *et al.*, 1983; Kaliner, 1984.) Thus, the possibility that leukotrienes may contribute to airways hyperreactivity has gained attention in recent studies which have provided experimental evidence for supra-additive interactions of leukotrienes with: histamine *in vitro* (Creese & Bach, 1983; Lee *et al.*, 1984) in the guinea-pig; histamine *in vivo* in the guinea-pig (Stewart *et al.*, 1983); 5-hydroxytryptamine (5-HT) *in vivo* in the cat (Holroyde & Jackson, 1983); prostaglandin $F_{2\alpha}$ in human volunteers (Barnes *et al.*, 1984).

Although there is considerable evidence that intravenously administered leukotrienes elicit bronchoconstriction by generating the release of cyclo-

[1] Present address and address for correspondence: Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London, WC2A 3PN

oxygenase products (Vargaftig *et al.*, 1981; Folco *et al.*, 1982; Piper & Samhoun, 1982), when administered by aerosol, they appear to elicit a direct bronchoconstrictor effect (Hamel *et al.*, 1982; Dahlen, 1983; Leitch *et al.*, 1983).

In the present study the effects of intravenous and aerosol administration of LTC_4 and LTD_4 on the bronchoconstrictor potency of histamine have been examined.

Methods

Isolated airways smooth muscle preparations

Guinea-pigs (Dunkin-Hartley) of either sex, weighing between 350 and 450 g, were killed by a blow to the head and the trachea was rapidly removed and placed in Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgCl_2$ 0.5, NaH_2PO_4 1.0 and D-(+)-glucose 11.1. The Krebs solution used during dissection, and for bathing the preparation in the organ bath, was maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Tracheal strips were prepared according to the method of Constantine (1965) as modified by Emmerson & Mackay (1979). Each trachea was bisected, giving strips from both the laryngeal and bronchial ends, which were randomly allocated to treatments. Each strip was mounted in a 25 ml organ bath under a load of 0.5 g and equilibrated for 60 min, during which time the bathing solution was exchanged every 15 min and a stable baseline developed. Responses were measured by use of Ugo Basile isotonic transducers (Model No. 7006) and displayed on a 4-channel Grass Polygraph (Model No. 79D) or a Rikadenki Chart Recorder (R-O2). Following equilibration, all strips were exposed to a supramaximal concentration of acetylcholine (0.5 mM) after which a washout period of 60 min was allowed. Contractile responses to histamine are expressed as a percentage of the response to the standardizing concentration of acetylcholine. LTC_4 (2 or 10 nM) or an equal volume of Krebs solution was added to either the bronchial or laryngeal end of the trachea before starting a cumulative histamine concentration – response curve.

Measurements of airways function in vivo

Guinea-pigs, weighing between 350 and 550 g, were anaesthetized with a mixture of sodium pentobarbitone (0.3% w/v) and urethane (25% w/v) at a dose of 4 to 5 ml kg^{-1}, i.p. A tracheal cannula, through which the animals were mechanically ventilated, was then inserted. The ventilation pump (Palmer) was adjusted to deliver 0.7 ml of air per 100 g body weight per stroke at a stroke rate of 60 min^{-1}. Blood pressure, measured from the right common carotid artery, was monitored on a Grass Polygraph (Model 79D). Heart rate was derived from the blood pressure signal using a tachograph. Intravenous injections were given in a volume less than 0.5 ml kg^{-1}, via a cannula inserted in the left jugular vein, after which the cannula was flushed with 0.2 ml saline. Following surgical preparation, gallamine triethiodide (4 mg kg^{-1}, i.v.) was administered to prevent spontaneous respiratory movements.

Aerosols were generated from a Hudson nebulizer at a flow rate of 6 l min^{-1}. The resulting aerosol was introduced into the inspiratory line at normal airflow rate and tidal volume between the pump and the tracheal cannula. Aerosols were generated from solutions of saline containing (0–16 μM leukotriene) for a fixed period of 30 s. In experiments in which the interaction between intravenously administered leukotriene and histamine was examined, leukotrienes were injected 20 s before histamine since this pretreatment time had previously been determined to be optimal for LTD_4 (Stewart *et al.*, 1984a). It was established in pilot experiments that saline administered 20 s before histamine had no effect on the ensuing bronchoconstrictor responses. Responses to histamine were elicited at intervals of not less than 6 min.

Breath to breath changes in airways resistance (R_L) and dynamic compliance (C_{dyn}) were measured according to the principles of Amdur & Mead (1958) as modified for anaesthetized guinea-pigs by Diamond (1972). Animals were placed in a perspex whole body plethysmograph. Transpulmonary pressure (TPP) was measured by attaching one port of a Pye differential pressure transducer to the T-junction on the tracheal cannula and the other port to the interior of the plethysmograph. This method of measurement of TPP is an approximation to the more common but more invasive method of taking the difference in pressure between the tracheal cannula and a cannula placed in a pleural cavity created by pneumothorax. Nevertheless, the method used in the present study has been validated by Diamond (1972) in the guinea-pig. Inspiratory and expiratory airflow rates (Q) were measured across a pneumotachograph attached to the rear of the plethysmograph at one end to a reservoir of 4 l at the other end to ensure that the differential pressure across the pneumotachograph was linear over the range of Q. The pneumotachograph consisted of a short piece of copper tubing filled with 19G needle shafts to act as an impedance. The difference in pressure across the impedance of the pneumotachograph was measured by attachment of sidearms to the ports of a Statham differential pressure transducer. The inspiratory airflow rate was electronically integrated to give tidal volume. Following preamplification by a Grass Polygraph (79D), the signals from the

differential pressure transducers were fed into a modified EAI 180 analogue-hybrid computer for the on-line computation of R_L and C_{dyn} according to a modification of the programme developed by Mindlin (1969). In all *in vivo* experiments, recovery from bronchoconstriction was facilitated by positive pressure re-inflation of the lungs.

Analyses of data

Responses to histamine following leukotriene (i.v.) pretreatment were preceded and followed by bracket control responses to histamine alone. The interaction between histamine and leukotriene was considered to be overadditive when the magnitude of the response to histamine following leukotriene pretreatment was significantly greater ($P < 0.05$, paired Student's t test) than the addition of the magnitudes of the response to leukotriene alone and the mean of the bracket control histamine responses.

Contractile responses to histamine in the isolated trachea were measured from the baseline prior to the start of the cumulative concentration-response curve. Histamine concentration-response lines obtained on the isolated trachea were analysed by linear regression of log concentration on responses between 20 and 80% of the maximal response obtained in individual experiments. The log of the concentration producing 50% of the maximal response (log EC_{50}) was obtained and the results from six experiments were pooled to give a geometric mean with a standard error of the mean (s.e.mean). Significant differences ($P < 0.05$) were determined by the unpaired Student's t test.

Reactivity to histamine *in vivo* was determined by estimation of the log of the dose required to increase airways resistance by 100% (log $ED_{100}R_L$) or decrease dynamic compliance by 50% (log $ED_{50}C_{dyn}$). These values were determined by obtaining bronchoconstrictor responses less than and greater than 100% increases in R_L, respectively, which necessitated using doses of histamine in the range 9–36 mol kg^{-1}. These responses corresponded to decreases of C_{dyn} of less than and greater than 50%. A regression line for the log of the dose on the response was calculated and the log $ED_{100}R_L$ and log $ED_{50}C_{dyn}$ values were obtained by interpolation.

A treatment period between 20 and 60 min followed, after which the log $ED_{100}R_L$ and log $ED_{50}C_{dyn}$ values were again determined. Data are presented as the mean change (Post- minus Pre-aerosol) in log $ED_{100}R_L$ or log $ED_{50}C_{dyn}$ with associated standard errors of the means.

This method of analysis was chosen since a linear relationship between the log doses of histamine and responses was observed for both R_L ($r = 0.54$, $P < 0.001$, $n = 75$) and C_{dyn} ($r = 0.63$, $P < 0.001$, $n = 105$).

Analyses of leukotrienes

The nominal concentrations of solutions of LTC_4 and LTD_4 were confirmed by u.v. spectrophotometry (Hitachi UV Spectrophotometer) by relating the absorbance at 280 nm to that indicated by the information accompanying each shipment. In addition, a spectral scan was routinely performed to ensure that the u.v. spectra corresponded to those published for LTC_4 and LTD_4.

Drugs

All reagents were of analytical grade: gallamine triethiodide (May & Baker); histamine diphosphate, urethane (Sigma Chemical Co.); leukotrienes C_4 and D_4 (Merck Frosst Laboratories, Quebec, Canada, or Calbiochem); sodium pentobarbitone (Abbott).

Results

Effect of leukotriene C_4 on histamine-induced contractile responses in vitro

Exposure of the isolated trachea to LTC_4 (2 nM) had no detectable effect on the sensitivity or the maximum response to that produced by histamine (Table 1). At a concentration of 10 nM, LTC_4, which elicited a contractile response approximately half that of the maximum response to histamine, caused a significant ($P < 0.05$, unpaired Student's t test) rightward shift in the concentration-response curve.

In addition, the maximum response to histamine (alone) was significantly reduced ($P < 0.05$, unpaired Student's t test) in the presence of 10 nM LTC_4, although the sum of the histamine maximum response and the LTC_4 response was not significantly different ($P > 0.05$, unpaired Student's t test) to the maximum response to histamine in paired control (Krebs-treated) preparations (Table 1).

Histamine-induced bronchoconstriction: effect of leukotriene C_4 or D_4 pretreatment

The resting values of R_L and C_{dyn} were 0.32 ± 0.01 cmH_2O ml^{-1} s^{-1}, $n = 72$ and 0.57 ± 0.03 ml cmH_2O^{-1}, $n = 72$, respectively.

LTD_4 (0.05–0.20 nmol kg^{-1}, i.v.), but not equibronchoconstrictor doses of LTC_4 (0.1–0.4 nmol kg^{-1}, i.v.), significantly enhanced the histamine (9–36 nmol kg^{-1}, i.v.)-induced increases in airways resistance in a dose-dependent manner (Figure 1, Table 2).

Furthermore, at pretreatment doses of 0.1 nmol kg^{-1}, i.v., LTC_4 and LTD_4 elicited direct bronchoconstrictor responses that were indistingui-

Table 1 The effects of leukotriene C_4 (LTC_4) on the sensitivity and maximum contractile response to histamine of the guinea-pig isolated trachea

Pretreatment	n[1]	Histamine (− log EC_{50})	Maximum response[2] (response to LTC_4)
Krebs	6	5.266 ± 0.068	96.6 ± 11.8
LTC_4 2 nM	6	5.252 ± 0.042	103.2 ± 9.5 (8.4 ± 2.6)
Krebs	6	5.458 ± 0.019	111.8 ± 9.5
LTC_4 10 nM	6	5.134 ± 0.066*	108.7 ± 3.2 (56.4 ± 4.9)

[1] n = number of observations. Values are mean ± s.e.mean.
[2] Maxima are presented as percentages of the response to 0.5 mM acetylcholine.
* $P < 0.05$, unpaired Student's t test.

shable from each other. The magnitudes of the histamine-induced bronchoconstrictions used to test for augmentation at the pretreatment doses of leukotrienes of 0.1 nmol kg^{-1}, i.v., were also indistinguishable. Nevertheless, the difference between the increase in R_L elicited by the combination of LTD_4 and histamine was significantly greater ($P < 0.05$, unpaired Student's t test) than that of the combination of LTC_4 and histamine (Table 2).

Effects of aerosolized leukotrienes on airways reactivity to histamine

LTC_4 (1–16 μM, 30 s) and LTD_4 (1–16 μM, 30 s) each elicited concentration-dependent increases in R_L and decreases in C_{dyn} (Table 3) which were maximal within 5 min and subsided over a 20 min period (Table 4). At 4 μM, LTC_4 induced a bronchoconstriction greater than that of LTD_4. Reactivity to histamine (4.5–36 nmol kg^{-1}, i.v.) was determined before and up to 60 min after exposure to leukotriene aerosols at 20 min intervals (Figure 2). The log shifts in the histamine dose-response curve determined 20 min after aerosol indicate that both LTD_4 (1–16 μM) and LTC_4 (4 and 16 μM) aerosols significantly increase the reactivity of the central airways to histamine (reflected by changes in R_L, Table 5). In contrast, histamine reactivity was significantly increased on peripheral airways (reflected by changes in C_{dyn}) only at the leukotriene concentration of 4 μM.

There was no significant correlation between the initial reactivity to histamine and the magnitude of the shift induced when the data for 4 and 16 μM were pooled for either R_L (LTC_4, $r = 0.25$, $n = 9$, $P > 0.05$; LTD_4, $r = 0.17$, $n = 10$, $P < 0.05$) or C_{dyn} (LTD_4, $r = 0.40$, $n = 10$, $P > 0.05$).

However, there was a significant correlation between initial reactivity to histamine and the shift in log C_{dyn} 50 elicited by LTC_4 ($r = 0.72$, $n = 9$, $P < 0.05$). There was also no correlation between the bronchoconstriction induced by the leukotrienes at 4 and 16 μM and the log shifts for either R_L (LTC_4, $r = 0.40$, $n = 9$, $P > 0.05$; LTD_4, $r = 0.003$, $n = 10$, $P > 0.05$) or C_{dyn} (LTC_4, $r = 0.35$, $n = 9$, $P > 0.05$; LTD_4, $r = 0.40$, $n = 10$, $P > 0.05$). It is of interest to note that the 1 μM aerosol of LTD_4 which had no detectable bronchoconstrictor effect caused a small but nevertheless significant increase ($P < 0.01$, unpaired Student's t test) in the potency of histamine on R_L.

The time course of the potentiating actions of LTC_4 (4 μM) and LTD_4 (4 μM) was investigated up to 60 min after exposure to aerosol (Figure 3). At 20, 40 and 60 min post-aerosol, LTC_4 significantly increased the potency of histamine on R_L, whereas LTD_4 potentiated histamine at 20 and 40 min but the shift obtained was not significantly different from that obtained in saline-exposed animals at 60 min.

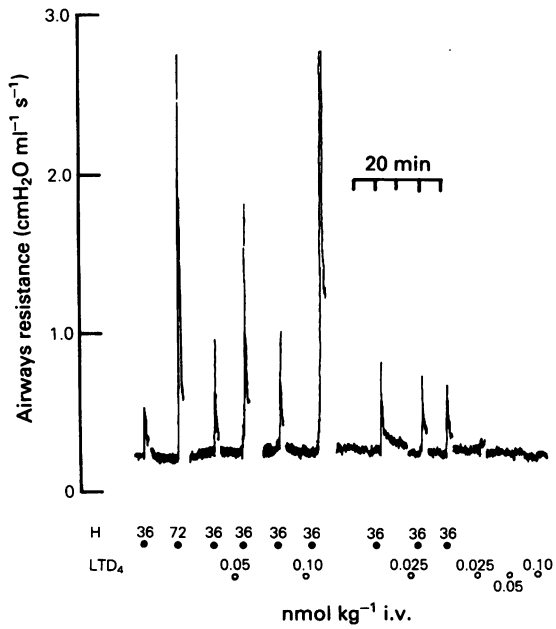

Figure 1 The effect leukotriene D_4 (LTD_4, 0.025–0.100 nmol kg^{-1}, i.v.) pretreatment on histamine (36 nmol kg^{-1}, i.v.)-induced increases in airways resistance in anaesthetized, mechanically ventilated guinea-pigs. The effect of LTD_4 is compared to that of bracket control responses to histamine. The doses of LTD_4 used had little effect on airways resistance when injected alone.

Table 2 The effects of intravenously administered leukotriene C_4 (LTC_4) or LTD_4 on histamine (9–36 nmol kg^{-1}, i.v.)-induced increases in airways resistance (R_L)

Pretreatment (nmol kg^{-1})	*Treatment*	n	*Increase in* R_L ($cmH_2O\ ml^{-1}\ s^{-1}$)	*Mean difference*[1]
—	Histamine	16	0.536 ± 0.060	
LTD_4 0.05	—	16	0.054 ± 0.019	
LTD_4 0.05	Histamine	16	1.029 ± 0.141	+ 0.440 ± 0.128*
—	Histamine	16	0.601 ± 0.092	
LTD_4 0.10	—	16	0.116 ± 0.028	
LTD_4 0.10	Histamine	16	1.841 ± 0.211	+ 1.137 ± 0.196**
—	Histamine	6	0.505 ± 0.086	
LTD_4 0.20	—	6	0.245 ± 0.080	
LTD_4 0.20	Histamine	6	2.322 ± 0.231	+ 1.571 ± 0.170**
—	Histamine	4	0.628 ± 0.120	
LTC_4 0.10	—	4	0.118 ± 0.013	
LTC_4 0.10	Histamine	4	0.897 ± 0.051	+ 0.151 ± 0.109
—	Histamine	3	1.301 ± 0.130	
LTC_4 0.20	—	3	0.088 ± 0.010	
LTC_4 0.20	Histamine	3	1.598 ± 0.317	+ 0.210 ± 0.207
—	Histamine	3	1.937 ± 0.225	
LTC_4 0.40	—	3	0.175 ± 0.020	
LTC_4 0.40	Histamine	3	2.065 ± 0.387	− 0.047 ± 0.327

*$P < 0.01$; **$P < 0.001$, paired Student's t test for comparison of the addition of the increases in R_L elicited by the separate administration of histamine and LTC_4 or LTD_4 and that elicited by the combined administration of these bronchoconstrictors

[1]The mean difference represents the increase in R_L elicited by histamine, 20 s after pretreatment with either LTD_4 or LTC_4, and that elicited by their separate administration.

Discussion

Either LTC_4 or LTD_4, administered by aerosol, increases the bronchoconstrictor potency of histamine in a concentration-dependent manner. LTC_4, as has been previously reported for LTD_4 (Stewart *et al.*, 1983), did not enhance histamine-induced contractions of airways smooth muscle. LTD_4 dose-dependently enhanced histamine-induced increases in R_L, confirming earlier findings (Stewart *et al.*, 1983; 1984a, b), whereas under the same conditions, LTC_4 did not possess a similar bronchoconstriction enhancing action.

Two previous studies have shown that leukotrienes enhance histamine-induced contractile responses of airways smooth muscle. This occurred (i) under conditions of lowered Ca^{2+} concentration (0.1 mM), but not where Ca^{2+} (2.5 mM) was used (Creese & Bach,

Table 3 Effects of aerosols of leukotriene C_4 (LTC_4) or LTD_4 on airways resistance (R_L) and dynamic compliance (C_{dyn})

Aerosol (μM)	n	R_L[1]	C_{dyn}[1]
Saline	15	101 ± 3	91 ± 2
LTC_4 1	4	121 ± 6*	80 ± 6*
LTC_4 4	3	215 ± 32*	46 ± 9*
LTC_4 16	6	388 ± 110*	37 ± 8**
LTD_4 1	14	107 ± 2	91 ± 2
LTD_4 4	5	125 ± 6**	75 ± 5**
LTD_4 16	5	222 ± 34*	50 ± 8*

[1]Data are presented as peak post-aerosol values as percentages of the values before aerosol treatment; mean ± s.e.mean values are shown.
*$P < 0.05$; **$P < 0.001$, unpaired Student's t test compared to saline aerosol-treated group.

Table 4 Time-course of leukotriene C_4 (LTC_4, 4 μM) and LTD_4 (4 μM) aerosol-induced increases in airways resistance (R_L)

Aerosol	n	R_L[1] *Time after aerosol* (min) 2	5	10	20
Saline	4	105 ± 4	105 ± 4	99 ± 3	97 ± 1
LTC_4 4μM	3	123 ± 12	189 ± 39	165 ± 29	123 ± 7
LTD_4 4μM	5	107 ± 2	123 ± 6	114 ± 6	106 ± 4

[1]Data are expressed as the post-aerosol values as percentages of the values before aerosol treatment.

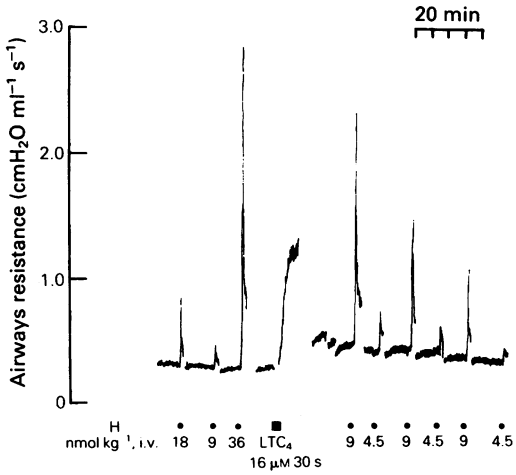

Figure 2 The effect of aerosolized leukotriene C_4 (LTC_4, 16 μM, 30 s) on sensitivity to histamine (4.5–36 nmol kg^{-1}, i.v.)-induced increases in airways resistance in anaesthetized, mechanically ventilated guinea-pigs. Recovery from the direct bronchoconstrictor effect of LTC_4 was facilitated by positive pressure reinflation of the lungs to maximum lung capacity at approximately 5 min intervals during the 20 min pretreatment period.

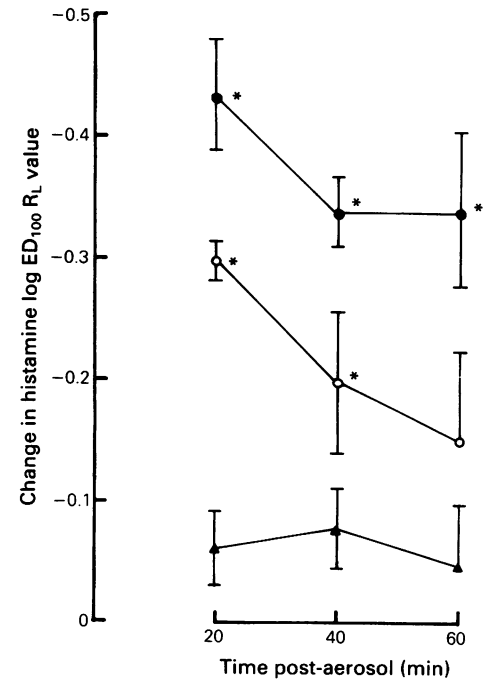

Figure 3 Time-course of the potentiation of histamine by leukotriene C_4 (LTC_4, 4 μM, 30 s, $n = 3$, ●) and LTD_4 (4 μM, 30 s, $n = 5$, ○) compared to saline (30 s, $n = 5$, ▲. Data are presented as the post-aerosol changes in log ED_{100} R_L values from those determined for histamine during the control period, i.e., before the respective aerosol treatments. *$P < 0.05$, unpaired Student's t test, comparing saline aerosol-treated guinea-pigs with those exposed to leukotrienes.

Table 5 Effects of aerosols of leukotriene C_4 (LTC_4) or LTD_4 on the potency of intravenously administered histamine on airways resistance (R_L) and dynamic compliance (C_{dyn}) determined 20 min after aerosolization of saline or leukotrienes

Aerosol treatment (μM)	n	*log shifts*[1] R_L[1]	C_{dyn}
Saline	15	− 0.038 ± 0.017	− 0.083 ± 0.037
LTC_4 1	4	− 0.062 ± 0.026	− 0.108 ± 0.055
LTC_4 4	3	− 0.433 ± 0.045**	− 0.333 ± 01074*
LTC_4 16	6	− 0.324 ± 0.045**	− 0.288 ± 0.102
LTD_4 1	14	− 0.140 ± 0.020**	− 0.139 ± 0.026
LTD_4 4	5	− 0.298 ± 0.016**	− 0.267 ± 0.050*
LTD_4 16	5	− 0.371 ± 0.050**	− 0.204 ± 0.059

[1] Data are presented as the change in the log of the dose of histamine (post-aerosol minus pre-aerosol) required to elicit a 100% increase in R_L or a 50% decrease in C_{dyn}. Values are mean ± s.e.mean. *$P < 0.05$; **$P < 0.01$, unpaired Student's t test compared to the shift obtained in the saline aerosol-treated group.

1983); (ii) following removal of, and recovery from, contractile concentrations of LTE_4 but not LTC_4 or LTD_4 (Lee *et al.*, 1984).

It may be concluded that, under physiological conditions, neither LTC_4 nor LTD_4 induces increased responsiveness to histamine on airways smooth muscle. However, the sensitivity of tracheal smooth muscle to histamine determined by Lee *et al.*, (1984) was considerably less (approx. 0.7 log units) that that determined in the present study and by other workers (cf. Drazen & Schneider, 1978; Schreurs & Nijkamp, 1984). Nevertheless, synergistic interaction between leukotrienes and histamine have been observed in cardiovascular smooth muscle (Hand *et al.*, 1983) and in cardiac muscle (Levi & Burke, 1980).

The higher pretreatment concentration of LTC_4 (10 nM), which elicited a response approximately equal to the contractile response at the EC_{50}, shifted the histamine concentration-response curve to the right. This observation suggests that LTC_4 and histamine

are not independent agonists i.e., there is an overlap in the mechanisms by which these agonist contract tracheal smooth muscle, as has been previously reported for acetylcholine and histamine in this preparation (Stewart *et al.*, 1984c).

In contrast to intravenously administered LTD_4, LTC_4 at equibronchoconstrictor doses did not augment histamine-induced increases in R_L. There is increasing pharmacological (Fleisch *et al.*, 1982; Gleason *et al.*, 1982; Lee *et al.*, 1984) and biochemical evidence (Hogaboom *et al.*, 1983; Cheng & Townley, 1984) of multiple receptors for the leukotrienes. The lack of enhancement by LTC_4 may be explained by the existence of a receptor for LTD_4 which is not activated by LTC_4. Alternatively, the abilities of LTC_4 and LTD_4 to elicit pulmonary vasoconstriction may differ as has been reported in the rat (Voelkel *et al.*, 1984) in which species, LTC_4, but not LTD_4, causes pulmonary vasoconstriction. LTC_4, by constricting the pulmonary artery and thereby reducing pulmonary blood flow, may reduce the access of histamine to airways smooth muscle. This action could be expected to mask an enhancement of the bronchoconstrictor response to histamine. Nevertheless, LTD_4 is reported to constrict guinea-pig isolated pulmonary arteries (Hand *et al.*, 1983) and may be equipotent with LTC_4 in this species (Hand *et al.*, 1981).

Both LTC_4 and LTD_4 aerosols potentiated histamine in a concentration-dependent manner, the effect of LTC_4 being of longer duration. The potentiation induced by LTC_4 or LTD_4 did not correlate with their direct bronchoconstrictor activity which was also concentration-dependent over the range of concentrations ($1-16\,\mu M$) tested. It appears that the enhancing action of the leukotrienes may be independent of their bronchoconstrictor activities. In addition, there was no detectable relationship between the preleukotriene sensitivity to histamine and the ability of either LTC_4 or LTD_4 to potentiate histamine on R_L. However, there was a negative correlation between the initial potency of histamine on C_{dyn} and the magnitude of the potentiation induced by LTC_4, but not that induced by LTD_4. Thus, there appears to be no consistent relationship between the initial potency of histamine on R_L or C_{dyn} and the magnitudes of potentiation induced by LTC_4 or LTD_4.

The bronchoconstrictor actions of intravenously administered leukotrienes are inhibited by cyclo-oxygenase inhibitors, whereas aerosolized leukotriene-induced bronchoconstriction is unaffected or enhanced (Hamel *et al.*, 1982; Dahlen, 1983; Leitch *et al.*, 1983) leading to the suggestion that the mechanism of bronchoconstriction may differ between these routes of administration.

Thus, the difference in activity of LTC_4 between these routes of administration may be related to differences in the involvement of cyclo-oxygenase products. Alternatively, it is possible that LTC_4, when administered by aerosol, is converted to LTD_4, as has been observed in the guinea-pig isolated ileum (Piper *et al.*, 1982), during the 20 min pretreatment period. As a consequence, the enhancement induced by aerosolized LTC_4 may be due to the formation of LTD_4. This suggestion is consistent with the longer duration of action of LTC_4 and its failure to enhance when administered intravenously 20 s before histamine, during which time little conversion could be expected. However, further studies using inhibitors of γ-glutamyltranspeptidase are required to determine whether aerosolized LTC_4 acts directly or via conversion to LTD_4.

Intravenously administered LTD_4 enhances histamine-induced bronchoconstriction by a mechanism which is, at least partly, dependent on vagal cholinergic nerves (Stewart *et al.*, 1984b). Whether a similar mechanism is responsible for the present observations on aerosolized LTC_4 and LTD_4 remains to be investigated. Nevertheless, there is evidence in support of the possibility that aerosolized leukotrienes also activate cholinergic nerves. It has been reported that aerosols of LTD_4 enhance 5-HT-induced bronchoconstriction in the cat by a vagal-dependent mechanism (Holyroyde & Jackson, 1983). Furthermore, the hyperreactivity of Basenji-Greyhounds to LTD_4 is reported to be blocked by atropine-pretreatment (Hirshman *et al.*, 1983).

The lack of an enhancing action of LTC_4 in the present study, or LTD_4 on contractile effects of histamine on the isolated trachea (Stewart *et al.*, 1983) suggests that aerosols of leukotrienes potentiated histamine *in vivo* by indirect mechanisms.

In conclusion, the present study provides evidence that is consistent with the hypothesis that leukotrienes may contribute to the regulation of airways reactivity to histamine in the guinea-pig. The previously reported failure of LTD_4 to enhance intravenously administered acetylcholine (Stewart *et al.*, 1984b) together with the failure of sub-threshold concentrations of leukotrienes to enhance histamine-induced bronchoconstriction in healthy human volunteers (Barnes *et al.*, 1984) preclude the possibility that leukotrienes are the sole, or even the major, mediators of the phenomenon of non-specific bronchial hyperreactivity.

The authors thank Dr J. Rokach (Merck Frosst Laboratories, Quebec, Canada) for the generous gifts of leukotrienes C_4 and D_4, and Prof. P.J. Piper for reviewing the manuscript. This work was supported, in part, by the Australian Tobacco Research Foundation.

References

ADAMS, G.K. & LICHTENSTEIN, L.M. (1979). *In vitro* studies of antigen-induced bronchospasm; effect of antihistamine and SRS-A antagonists on response of sensitized guinea-pig and human airways to antigen. *J. Immunol.*, **122**, 555–562.

AMDUR, M.O. & MEAD, J. (1958). Mechanics of respiration in unanaesthetized guinea-pigs. *Am. J. Physiol.*, **192**, 364–368.

BARNES, N.C., PIPER, P.J. & COSTELLO, J.F. (1984). Actions of inhaled leukotrienes and their interactions with other allergic mediators. *Prostaglandins*, **28**, 629–631.

CARTIER, A., THOMSON, N.C., FRITH, P.A., ROBERTS, R. & HARGREAVE, F.E. (1982). Allergen-induced increases in bronchial responsiveness to histamine; relationship to the late asthmatic response and change in airway caliber. *J. Allergy clin. Immunol.*, **70**, 170–177.

CHENG, J.B. & TOWNLEY, R.G. (1984). Identification of leukotriene D_4 receptor binding sites in guinea-pig lung homogenates using (^{3}H) leukotriene D_4. *Biochem biophys. Res. Comm.*, **118**, 20–26.

CONSTANTINE, J.W. (1965). The spirally cut tracheal strip preparation. *J. Pharm. Pharmac.*, **17**, 384–385.

CREESE, B.R. & BACH, M.K. (1983). Hyperreactivity of airways smooth muscle produced *in vitro* by leukotrienes. *Prostaglandins, Leuk. Med.*, **11**, 161–169.

DAHLEN, S-E. (1983). The signficance of liberated cyclooxygenase products for the pulmonary and cardiovascular actions of leukotriene C_4 in the guinea-pig depends upon the routes of administration. *Acta physiol. scand.*, **118**, 415–421.

DIAMOND, L. (1972). Potentiation of bronchomotor responses by beta adrenergic antagonists. *J. Pharmac. exp. Ther.*, **181**, 434–445.

DRAZEN, J.M. & SCHNEIDER, M.W. (1978). Comparative responses of tracheal spirals and parenchymal strips to histamine and carbachol *in vitro*. *J. clin. Invest.*, **61**, 1441–1447.

EMMERSON, J. & MACKAY, D. (1979). The zig-zag tracheal strip. *J. Pharm. Pharmac.*, **31**, 798.

EMPEY, D.W., LAITINEN, L.A., JACOBS, L., GOLD, W.M. & NADEL, J.A. (1976). Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. *Am. Rev. Resp. Dis.*, **113**, 131–139.

FLEISCH, J.H., RINKEMA, L.E. & BAKER, S.R. (1982). Evidence for multiple leukotriene D_4 receptors in smooth muscle. *Life Sci.*, **31**, 577–581.

FOLCO, G.C., OMINI, C., VIGANO, T., BRUNELLI, G., ROSSONI, G. & BERTI, F. (1982). Biological activity of leukotriene C_4 in guinea-pigs: In vitro and in vivo studies; In *Leukotrienes and Other Lipoxygenase Products.* ed. Samuelsson, B. & Paoletti, R. pp. 153–167. *Advances in Prostaglandin, Thromboxane, and Leukotriene Research.* New York: Raven Press.

GLEASON, J.G., KRELL, R.D., WEICHMAN, B.M., ALI, F.E. & BERKOWITZ, B. (1982). Comparative pharmacology and antagonism of synthetic leukotrienes on airway and vascular smooth muscle. In *Leukotrienes and Other Lipoxygenase Products.* ed. Samuelsson, B. & Paoletti, R. pp. 243–250. *Advances in prostaglandin, thromboxane and leukotriene research.* New York: Raven Press.

HAMEL, R., MASSON, P., FORD-HUTCHINSON, A.W., JONES, T.R., BRUNET, G. & PIECHUTA, H. (1982). Differing mechanisms for leukotriene D_4-induced bronchoconstriction in guinea-pigs following intravenous and aerosol administration. *Prostaglandins*, **24**, 419–432.

HAND, J.M., WILL, J.A. & BUCKNER, C.K. (1981). Effects of leukotrienes on isolated guinea-pig pulmonary arteries. *Eur. J. Pharmac.*, **76**, 439–442.

HAND, J.M., WILL, J.A. & BUCKNER, C.K. (1983). Enhancement by histamine of the contractile effects of leukotriene D_4 on extralobar pulmonary arteries isolated from the guinea-pig. *Eur. J. Pharmac.*, **87**, 341–343.

HIRSHMAN, C.A., DARNELL, M., BRUGMAN, T. & PETERS, J. (1983). Airway constrictor effects of leukotriene D_4 in dogs with hyperreactive airways. *Prostaglandins*, **25**, 481–490.

HOGABOOM, G.K., MONG, S., WU, H.L. & CROOKE, S.T. (1983). Peptidoleukotrienes: distinct receptors for leukotriene C_4 and D_4 in the guinea-pig lung. *Biochem. biophys. Res. Comm.*, **116**, 1136–1143.

HOGG, J.C. (1981). Bronchial mucosal permeability and its relationship to airways hyperreactivity. *J. Allergy clin. Immunol.*, **67**, 421–425.

HOLROYDE, M.C. & JACKSON, D.M. (1983). Effect of leukotrienes (LTs) on pulmonary function and lung irritant receptors in cats and dogs. *Br. J. Pharmac.*, **79**, 217P.

KALINER, M. (1984). Hypotheses on the contribution of late-phase allergic responses to the understanding and treatment of allergic diseases. *J. Allergy clin. Immunol.*, **73**, 311–315.

LEE, T.H., AUSTEN, K.F., COREY, E.J. & DRAZEN, J.M. (1984). Leukotriene E_4-induced airway hyperresponsiveness of guinea-pig tracheal smooth muscle to histamine and evidence for three separate sulfidopeptide leukotriene receptors. *Proc. natn. Acad. Sci. U.S.A.*, **81**, 4922–4925.

LEITCH, A.G., COREY, E.J., AUSTEN, K.F. & DRAZEN, J.M. (1983). Indomethacin potentiates the pulmonary response to aerosol leukotriene C_4 in the guinea-pig. *Am. Rev. Resp. Dis.*, **128**, 639–643.

LEVI, R. & BURKE, J.A. (1980). Cardiac anaphylaxis: SRS-A potentiates and extends the effects of released histamine. *Eur. J. Pharmac.*, **62**, 41–49.

MALO, P.E., WASSERMAN, M.A., GRIFFIN, R., LEONG, B.K.J. & POWELL, D.J. (1983). Enhanced bronchoconstriction responses to prostaglandin $F_{2\alpha}$ following inhalation of sulfur dioxide. *Prostaglandins*, **25**, 179–192.

MINDLIN, S. (1969). On-line computation of respiratory characteristics. *EAI Applications Reference Library Bulletin 953055.*

PIPER, P.J., LETTS, L.G., SAMHOUN, M.N., TIPPINS, J.R. & PALMER, M.A. (1982). Actions of leukotrienes on vascular, airway, and gastrointestinal smooth muscle. In *Leukotrienes and Other Lipoxygenase Products.* ed. Samuelsson, G. & Paoletti, R. pp. 169–181. *Advances in Prostaglandin, Thromboxane, and Leukotriene Research.* New York: Raven Press.

PIPER, P.J. & SAMHOUN, M.N. (1982). Stimulation of arachidonic acid metabolism and generation of thromboxane A_2 by leukotrienes B_4, C_4 and D_4 in guinea-pig lung *in vitro*. *Br. J. Pharmac.*, **77**, 267–275.

SAMUELSSON, B. (1983). Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science*, **220**, 568–575.

SCHREURS, A.J.M. & NIJKAMP, F.P. (1984). Bronchial hyperreactivity to histamine induced by Haemophilus influenzae vaccination. *Agents & Actions*, **15**, 211–215.

STEWART, A.G., THOMPSON, D.C. & FENNESSY, M.R. (1983). Interaction of leukotriene D_4 and histamine on bronchomotor tone in the guinea-pig. *J. Pharm. Pharmac.*, **35**, 755–756.

STEWART, A.G., THOMPSON, D.C. & FENNESSY, M.R. (1984a). Leukotriene D_4 potentiates histamine-induced bronchoconstriction in guinea-pigs. *Agents & Actions*, **15**, 146–152.

STEWART, A.G., THOMPSON, D.C. & FENNESSY, M.R. (1984b). Involvement of capsaicin-sensitive afferent neurones in a vagal-dependent interaction between leukotriene D_4 and histamine on bronchomotor tone. *Agents & Actions*, **15**, 500–508.

STEWART, A.G., THOMPSON, D.C. & FENNESSY, M.R. (1984c). Acetylcholine and histamine interact supra-additively on bronchomotor tone in guinea-pigs *in vivo*: A possible consequence of the relationship between airways resistance and calibre. *Archs int. Pharmacodyn.*, **273**, 33–46.

VARGFTIG, B.B., LEFORT, J. & MURPHY, R.C. (1981). Inhibition by aspirin of bronchoconstriction due to leukotrienes C_4 and D_4 in the guinea-pig. *Eur. J. Pharmac.*, **72**, 417–418.

VOELKEL, N.F., STENMARK, K.R., REEVES, J.T., MATHIAS, M.M. & MURPHY, R.C. (1984). Actions of lipoxygenase metabolities in isolated rat lungs. *J. appl. Physiol.*, **57**, 860–867.

(Received May 15, 1985.
Revised November 14, 1985.
Accepted November 27, 1985.)