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The contractile action of leukotriene B_4 in the guinea-pig lung involves a vascular component

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1 Leukotriene B_4 (LTB_4) is a potent leukocyte chemoattractant, acting on specific receptors, BLT receptors. The aim of this study was to examine the mechanism of action of LTB_4 in the guinea-pig lung, using strips of lung parenchyma (GPLP), spirals of trachea (GPT) and bronchus (GPB) and rings of pulmonary artery (GPPA). Mechanical responses were studied in organ baths, and mediator release was assessed using enzyme immuno assay.

2 LTB_4 induced similar contractions of GPLP and GPPA, whereas LTB_4 had only small contractile effects in GPT and GPB. In addition, the contractile response to LTB_4 was reproduced in the human pulmonary artery.

3 In the GPLP, the unselective BLT receptor antagonist ONO-4057 abolished the contractions induced by LTB_4, whereas the selective BLT_1 receptor antagonist U–75302 only partly inhibited the LTB_4-induced contractions. In the GPPA, both antagonists abolished the response to LTB_4.

4 The effect of LTB_4 in GPPA and GPLP was indirect and mediated by the release of thromboxane A_2 and histamine, as supported by selective pharmacologic interventions and measurements of thromboxane B_2 and histamine in the organ baths.

5 In conclusion, the results indicate a new biological function of LTB_4, namely to constrict isolated pulmonary arteries. Moreover, the findings suggest that the LTB_4-induced contractions of GPPA were mediated by a BLT_1 receptor, whereas BLT_2 receptor activation accounted for a major part of the contraction of GPLP, making the latter preparation a suitable assay for BLT_2 receptors.
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Keywords: Leukotriene B_4; guinea-pig lung parenchyma; pulmonary artery; vasoconstriction; BLT receptor; histamine; thromboxane A_2

Abbreviations: AUC, area under the curve; GPB, guinea-pig bronchus; GPLP, guinea-pig lung parenchyma; GPPA, guinea-pig pulmonary artery; GPT, guinea-pig trachea; HPA, human pulmonary artery; LTB_4, leukotriene B_4; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2

Introduction

Leukotriene B_4 (LTB_4) is a potent leukocyte chemoattractant and a mediator of inflammation (Ford-Hutchinson *et al*., 1980; Smith *et al*., 1980; Dahlén *et al*., 1981; Ng *et al*., 1991; Huang *et al*., 1998), initially isolated and purified from leukocytes by Borgeat *et al*. (1976). In addition, LTB_4 has been reported to contract the guinea-pig lung parenchyma (GPLP; Hansson *et al*., 1981; Lewis *et al*., 1981; Sirois *et al*., 1981a, b; 1982; Piper & Samhoun, 1982). Since the GPLP is considered to be a pharmacologic model of small airway reactivity (Drazen & Schneider, 1978), the findings with LTB_4 have been assumed to support a bronchoconstrictor potential of LTB_4. However, inhalation of LTB_4 by asthmatic or nonasthmatic humans has exclusively been associated with a chemotactic response and not with bronchoconstriction (Black *et al*., 1989; Sampson *et al*., 1997), and aerosol challenge with LTB_4 does not cause bronchconstriction in guinea-pigs (Dahlén, unpublished observations). Since LTB_4 contracts the GPLP in the nanomolar dose range, the aim of this study was to characterise this response in an attempt to differentiate the components of the lung that are involved in the LTB_4-induced response in the GPLP. Therefore, the effects of LTB_4 in the GPLP were compared with those obtained in two airway preparations from the same animal, namely guinea-pig trachea (GPT) and guinea-pig bronchus (GPB), and in one vascular preparation, the pulmonary artery (GPPA). The hypothesis was that there were biological effects of LTB_4 in the vasculature that had been previously overlooked.

Leukotrienes exert their actions *via* membrane-bound G protein-coupled receptors, consisting of two receptor subclasses, BLT receptors activated by LTB_4, and CysLT receptors activated by the cysteinyl-leukotrienes (Bäck, 2002; Brink *et al*., 2003). The BLT receptors consist of two receptor subtypes, BLT_1 and BLT_2, which can be pharmacologically recognised using receptor antagonists, where ONO-4057 has been reported to inhibit both BLT receptors, whereas U-75302 is a selective BLT_1 receptor antagonist (Lawson *et al*., 1989; Kishikawa *et al*., 1992; Yokomizo *et al*., 1997, 2000). In addition, the ω-oxidised metabolite of LTB_4, 20-COOH-LTB_4,

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has been reported to be a preferential BLT_1 receptor agonist (Wang *et al*., 2000). Using these tools, the receptors mediating the contractile effects of LTB_4 in the guinea-pig lung were assessed in the present study.

In the GPLP, it has been established that the release of thromboxane A_2 (TXA_2) mediates a major component of the myotropic response induced by LTB_4 (Sirois *et al*., 1981a, 1982; Piper & Samhoun, 1982; Austen *et al*., 1983; Dahlén *et al*., 1983). In addition, using a superfusion technique, it was observed that antihistamines inhibited, in particular, the early part of the response to LTB_4 (Dahlén *et al*., 1983), suggesting that a part of the contraction was caused by the release of histamine. In order to confirm and extend these observations, the characterisation of the response to LTB_4 in the investigated preparations included measurements of histamine and thromboxane in the tissue bath, as well as interventions with antihistamines, the cyclooxygenase inhibitor indomethacin and the selective TP receptor antagonist BAY u3405.

A preliminary report of these results has been communicated to the British Pharmacological Society (Sakata *et al*., 2001).

Methods

Tissue preparation

Male Dunkin Hartley guinea-pigs (300–450 g) were asphyxiated by CO_2 and bled. The lung parenchyma was cut parallel to the peripheral margins of the lobes into four strips, each having a cross-sectional area of approximately 10 mm^2, and a length of about 25 mm. The trachea and the bronchus were cut open helically at an angle of approximately 45° relative to the long axis. The right and left branches of the main pulmonary artery were cut into rings (internal diameters 2–3 mm).

Macroscopically normal human lung tissue was obtained from four patients (two male and two female, 56–68 years old) undergoing surgery for lung carcinoma. Intrapulmonary arteries were immediately dissected free from the surrounding tissue and cut into rings with a length of approximately 5 mm and an inner diameter of approximately 2 mm.

In some vascular rings, the endothelium was mechanically removed by gently rubbing the luminal surface with a metal forceps. Endothelium denudation was confirmed functionally (see below) and also histologically in sections of GPPA stained by haematoxylin and eosin at the end of the organ bath experiments.

The experiments were approved by the local ethics committees for animal (N317/98) and human (KS 00-267) experiments, respectively.

Tissue bath experiments

All preparations were placed in 5 ml organ baths containing Tyrode's solution (composition, mM: NaCl: 149.2;. KCl: 2.7; $NaHCO_3$: 11.9; $CaCl_2$: 1.8; $MgCl_2$: 0.5; NaH_2PO_4: 0.4 and glucose 5.5) gassed with 6.5% CO_2 in O_2 at 37°C. Resting tensions were kept at 10 mN in GPPA, GPT and GPB, and 4 mN in GPLP. Mechanical responses were recorded isometrically *via* Grass FT-03 force-displacement transducers connected to an EMKA data acquisition system.

The bath fluid was initially changed at 10 min intervals during a 60–90 min equilibration period. In the GPPA, noradrenaline (10 μM) was first added and at the plateau of the contraction, a cumulative concentration response curve for the relaxant effect of bradykinin (10 nM–1 μM) was established in order to assess functionally the integrity of the endothelium. In the GPLP, GPT and GPB, tissue reactivity was initially assessed by cumulative challenge with histamine (1–30 μM). Concentration response curves as well as assessments of the effects of different interventions were derived from experiments where each preparation was exposed to LTB_4 only once because of previously demonstrated tachyphylaxis to LTB_4 (Dahlén *et al*., 1983). Drugs were administered 30 min prior to the application of LTB_4.

At the end of the experimental protocol, a maximal contraction was evoked by histamine (1 mM), acetylcholine (1 mM) and KCl (40 mM) in the GPLP, GPT and GPB, and by addition of KCl (40 mM) in the GPPA. After the experiments, the wet weights of the preparations were determined after blotting on a filter paper.

Measurement of thromboxane B_2 and histamine

For measurement of mediators in the bath fluid, 120 μl aliquots were withdrawn 15 min prior to the administration of LTB_4 and at the indicated times thereafter. Thromboxane B_2 (TXB_2) and histamine were measured using enzyme immunoassay kits from Cayman Chemical Company (Ann Arbor, MI, U.S.A.) and Immunotech (Marseille, France), respectively. The TXB_2 assay had a threshold corresponding to about 0.40 pM (15 pg ml^{-1}) bath concentration of TXB_2 and the data derived from dilutions in the linear portion of the assay curve (between 0.5 and 3 pM). For this antibody, the crossreactivity with 2,3-dinor-TXB_2 is 8.2%, whereas all other tested prostanoids have crossreactivities of less than 0.5%. The histamine measurements had a threshold of around 0.5 nM bath fluid concentration. The crossreactivities with methyl-histamine, histidine and serotonin were less than 0.05%.

Drugs and substances

Noradrenaline, acetylcholine, histamine, indomethacin, mepyramine and bradykinin were obtained from Sigma (St Louis, MO, U.S.A.). LTB_4 and 20-COOH-LTB_4 were from Cascade Biochemicals (Reading, U.K.), and Cayman Chemicals (Ann Arbor, MI, U.S.A.). The following drugs were kindly provided by the respective pharmaceutical company, BAYu3405 (3R-3-[4-fluorophenylsulphonamide]-1,2,3,4-tetrahydro-9-carbazolepropanoic acid) from Bayer AG (Leverkusen, Germany), metiamide from SKB (Swedeland, PA, U.S.A.), U-75302 (6-[6-{3-hydroxy-1 *E*,5*Z*-undecadienyl}-2-pyridinyl]-1,5-hexanediol) from PhamaciaUpjohn Co. (Kalamazoo, MI, U.S.A.), and ONO-4057 (5-[2-(2-carboxyethyl)-3-{6-(4-methoxyphenyl)-5*E*-hexenyl} oxyphenoxy] valeric acid) from ONO Pharmaceutical Co., Ltd. (Osaka, Japan).

Noradrenaline, acetylcholine, histamine, mepyramine, metiamide and bradykinin were dissolved in Tyrode's solution. ONO-4057 was dissolved in dimethylsulphoxide. BAYu3405 and U-75302 were dissolved in ethanol. Indomethacin was dissolved in 10% ethanol and 10% 1 M Tris (pH 8.0) in distilled water. In total, 5 μl of each stock solution, except for

KCl solution (100 μl), was administered into the 5 ml bath. The final concentrations of ethanol, methanol or dimethylsulphoxide in the bath were always below 0.1%.

Stock solutions of LTB_4 and 20-COOH-LTB_4 were dissolved in ethanol and methanol, respectively. The concentrations of the leukotriene solutions were determined each experimental day by UV spectrometry using the extinction coefficient 55000.

Data analysis

All results are expressed as mean ± s.e.m. Contractions are expressed as per cent of the final maximal contraction and as the area under the contraction-time curves (AUC), compared as either AUC of the whole responses ($AUC_{0-10\,min}$) or the AUC of the first phase of the contraction ($AUC_{0-1\,min}$). Measurements of TXB_2 and histamine were expressed as molar release per mg tissue wet weight. Statistical analysis was performed by using Student's *t*-test, Dunnett's test or Tukey test, as appropriate. A *P*-value of less than 0.05 was considered significant.

Results

Profile of agonist activities

In the GPLP, LTB_4 (1–1000 nM), 20-COOH-LTB_4 (10–1000 nM) and U-75302 (10–10,000 nM) induced concentration-dependent contractions (Figure 1a). In contrast, in the two airway preparations, LTB_4 had only a small contractile effect (Figure 1b). In the GPPA, LTB_4 induced concentration-dependent contractions, whereas U-75302 induced only small contractions of this preparation (Figure 1c).

Antagonism of LTB_4-induced contractions

Pretreatment with LTB_4 (10 nM) desensitised the GPLP to subsequent challenge with LTB_4 (100 nM), as shown by representative tracings in Figure 2. In preparations previously exposed to LTB_4 10 nM (by itself causing an initial response of 15.6 ± 7.6%, $n = 3$), the contraction induced by LTB_4 100 nM was 4.2 ± 2.0% ($n = 3$), as compared with 39.5 ± 3.3% in parallel control strips from the same animals ($P < 0.05$; Student's *t*-test). The partial agonist U-75302 (1 μM) induced contractions of the GPLP that were similar to those induced by LTB_4 (10 nM), but in contrast, this pretreatment caused only a partial inhibition of the contractile response to LTB_4 in the GPLP (Figures 2 and 3a).

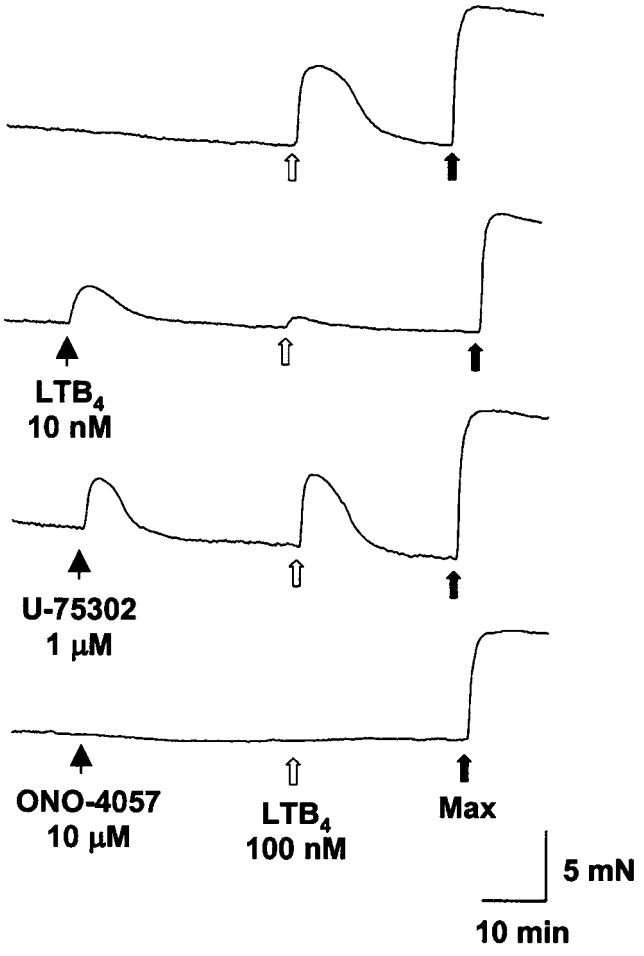

Figure 2 Representative tracings of the contractions induced by LTB_4 (100 nM) in the GPLP.

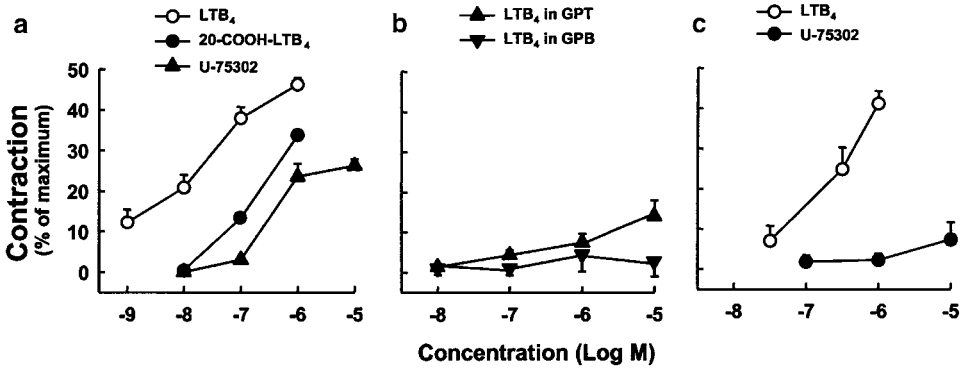

Figure 1 Concentration–response curves for (a) LTB_4 ($n = 5$), 20-COOH-LTB_4 ($n = 3$) and U-75302 ($n = 4$) in the GPLP, (b) LTB_4 in the GPT ($n = 4$–7) and GPB ($n = 4$–8), and (c) LTB_4 ($n = 4$–8) and U-75302 ($n = 4$) in the GPPA. Contractions (mean ± s.e.m.) are expressed as per cent of maximal contraction.

In the GPLP, the BLT_1 receptor partial agonist U-75302 inhibited the LTB_4-induced contractions concentration-dependently at lower concentrations (0.01–0.1 μM), whereas a higher concentration (1 μM) of U-75302 did not further inhibit the contractions (Figure 3a). In contrast, the unselective BLT receptor antagonist ONO-4057 (10 μM) almost abolished the contractions induced by LTB_4 (100 nM) in the GPLP (Figures 2 and 3b). The inhibition by ONO-4057 appeared concentration-dependent with less antagonism exerted at a lower concentration (1 μM; Figure 3b).

In contrast to the results obtained with LTB_4, the contractions induced by 20-COOH-LTB_4 (1 μM) were almost completely inhibited by U-75302 (1 μM) in the GPLP (Figure 3c).

Also in the GPPA, tachyphylaxis to repeated administration of LTB_4 was observed. A second application of LTB_4 (1 μM), 30 min after the first application of LTB_4 (1 μM), did not induce any contractions of the GPPA (first: $28.8 \pm 3.7\%$, second: $0.6 \pm 0.3\%$, $n=4$, $P<0.05$; Student's t-test). This tachyphylaxis was seen independent of whether or not the preparations were washed before the repeated application. In the GPPA, either ONO-4057 (0.1–10 μM) or U-75302 (0.01–1 μM) displayed concentration-dependent inhibition of the contractions induced by LTB_4 (1 μM) with complete antagonism observed at the highest concentrations tested (Figure 3d and e).

Effects of LTB_4 in human pulmonary artery

As indicated by representative tracings in Figure 4, LTB_4 (1 μM) induced a distinct short-lasting contraction of the human pulmonary artery (HPA). This contraction was $19.9 \pm 10.1\%$, $n=4$. The LTB_4-induced contraction of the HPA was abolished by either ONO-4057 (10 μM) or U-75302 (10 μM; Figure 4).

Effects of antihistamines and TP receptor antagonism in the guinea-pig preparations

The time course of the contractions induced by LTB_4 in the GPLP and GPPA, in the absence and presence of different drug treatments, is displayed in Figure 5.

In. the GPLP, either the TP receptor antagonist BAYu3405 (3 μM; Figure 5a) or the cyclooxygenase inhibitor indomethacin (10 μM; Figure 5b) significantly inhibited the contractions induced by LTB_4 (100 nM; Table 1). The combination of the H_1 receptor antagonist mepyramine (1 μM) with the H_2 receptor antagonist metiamide (1 μM) did not significantly alter the overall contraction ($AUC_{0-10\,min}$), but significantly inhibited the $AUC_{0-1\,min}$, that is, the first part of the contraction (Figure 5a and b; Table 1). In the presence of the combination of the antihistamines with either indomethacin or BAY u3405, the initial phase of the response was abolished (Figure 5a and b; Table 1). However, in the presence of the BLT_1 receptor antagonist U-75302 (1 μM), the antihistamines did not have any significant effect on either phase of the contraction (Figure 5c; Table 1).

In the GPPA, either BAYu3405 (3 μM) or the combination of mepyramine (1 μM) with metiamide (1 μM) caused a significant inhibition of the contractions ($AUC_{0-5\,min}$) induced by LTB_4 (1 μM; Figure 5d), whereas only the antihistamines significantly inhibited the early phase of the contraction ($AUC_{0-1\,min}$; Table 1). In addition, the combination of BAYu3405 with the antihistamines completely abolished the LTB_4-induced contractions of the GPPA (Figure 5d).

Measurements of thromboxane B_2 and histamine in the tissue bath

The concentrations of TXB_2, which is the stable metabolite of TXA_2, were significantly increased after stimulation of the

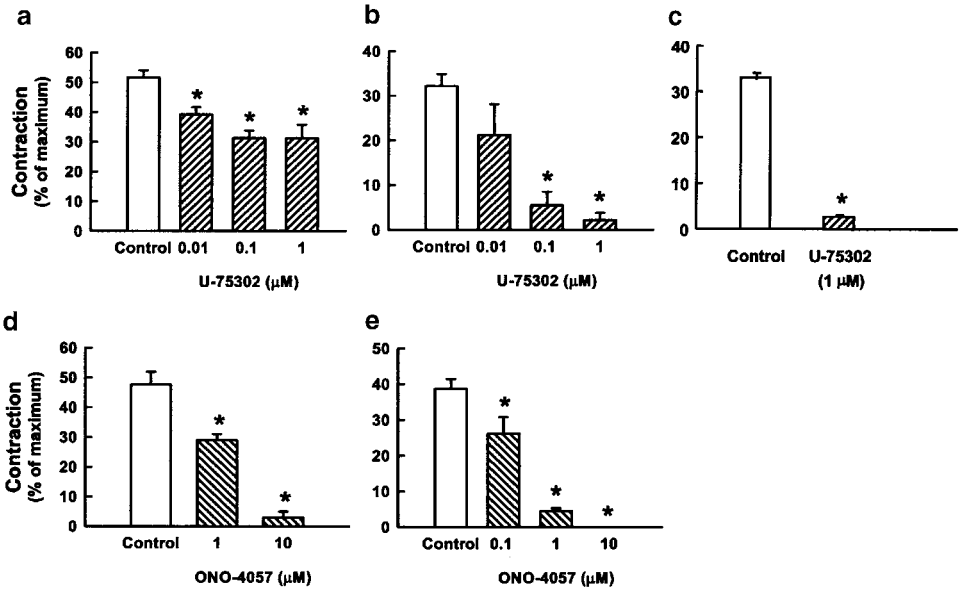

Figure 3 Effects of the BLT receptor antagonists ONO-4057 and U-75302, in the GPLP (a–c) and GPPA (d, e), on the contractions induced by either LTB_4 (a, b and d, e) or 20-COOH-LTB_4 (c). In the GPLP, LTB_4 was added at a concentration of 100 nM (a, b) and 20-COOH-LTB_4 at a concentration of 1 μM (c), whereas in the GPPA, LTB_4 was added at a concentration of 1 μM (d, e). Each bar represents the mean $\pm$ s.e.m. of 4–5 (a), 4–5 (b), three (c), 4–8 (d) and 5–8 (e) experiments. $^*P<0.05$ vs control, Dunnett's test (a, b, d and e) and Student's t-test (c).

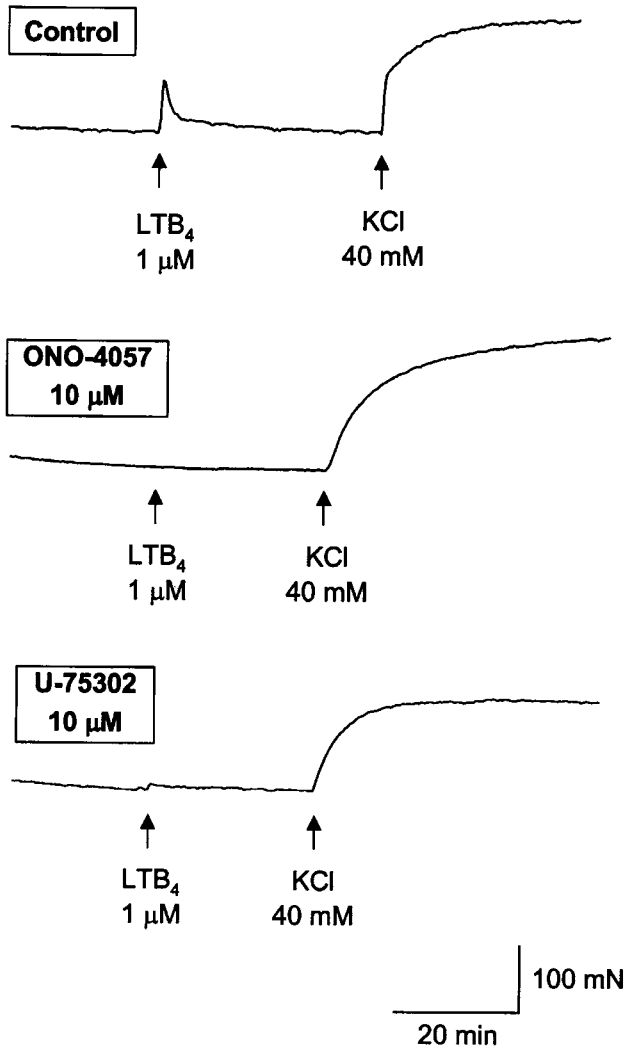

Figure 4 Representative tracings of the contractions induced by LTB_4 (100 nM) in the human pulmonary artery, and the effects of ONO-4057 (10 μM) and U-75302 (10 μM).

GPLP (Figure 6a) and GPPA (Figure 6b) with LTB_4 (100 nM and 1 μM, respectively). Also, the concentrations of histamine were significantly increased in both preparations after exposure to LTB_4 (Figure 6c and d).

Effect of endothelium denudation in the guinea-pig pulmonary artery

Endothelium denudation did not significantly alter the contraction induced by LTB_4 (1 μM) in the GPPA, but abolished the relaxation induced by bradykinin (1 μM) on noradrenaline (10 μM) precontracted preparations (Figure 7).

Discussion

In the present study, the contractile effect of LTB_4 on isolated GPLP was confirmed, and in addition, it was discovered that LTB_4 contracted pulmonary arteries from human and guinea-pig lungs, which has not previously been observed. The lung parenchyma preparation was originally introduced as a model for peripheral airway function (Lulich *et al*., 1976), which has also been suggested using lung preparations from guinea-pigs (Drazen & Schneider, 1978). However, in the present study, the contractile effects of LTB_4 in the GPLP were reproduced in the pulmonary artery, whereas either the trachea or bronchi exhibited only small contractions in response to LTB_4, raising a doubt as to the relative importance of airways in response to LTB_4 in the GPLP.

Interestingly, isolated superfused human lung parenchyma has been shown to contract in response to LTB_4, whereas LTB_4 showed little activity on human bronchi in the same study (Sirois *et al*., 1981b). The latter report is thus in line with the findings of the present study in the guinea-pig lung. In addition, since a contractile effect of LTB_4 was observed in the human pulmonary artery, it may be anticipated that the vasculature may be the main target for LTB_4 also in the human lung. In contrast to the results of the present study, the human pulmonary artery has previously been reported to be unresponsive to LTB_4 (Schellenberg & Foster, 1984). There is, however, one major experimental difference between that study and the present, namely that a cumulative addition of LTB_4 was performed in the previous study (Schellenberg & Foster, 1984), which may lead to receptor desensitisation. The BLT receptor is rapidly desensitised by its ligand, and Gaudreau *et al*. (2002) recently showed that this desensitisation involves the cytoplasmic tail of the BLT_1 receptor and phosphorylation of threonine (Thr^{308}). In the present study, a second administration of LTB_4 did not induce contractions of either GPLP or GPPA, indicating that a noncumulative dosing of LTB_4 must be used in order to avoid tachyphylaxis, and this is presumably the reason for the inability of previous studies to detect an LTB_4-indued pulmonary arterial constriction.

In the GPLP, the selective BLT_1 receptor partial agonist U-75302 (Yokomizo *et al*., 2000; Wang *et al*., 2000) had a significant contractile activity, but only partially inhibited the contractions induced by LTB_4. Moreover, the preferential BLT_1 receptor agonist 20-COOH-LTB_4 (Wang *et al*., 2000) induced contractions that were abolished by U-75302. Taken together, these results suggest a functional BLT_1 receptor in the GPLP. However, a major part of the LTB_4-induced contraction of the GPLP was resistant to U-75302, whereas the unselective BLT receptor antagonist, ONO-4057 (Yokomizo *et al*., 2000), abolished the contraction, indicating that a significant component of the LTB_4-induced contractions of the GPLP were mediated *via* a BLT_2 receptor. This is, to the best of our knowledge, the first physiological response that has been associated with a BLT_2 receptor. In view of these findings, it is of interest that BLT_2 receptor mRNA has been reported to be expressed in the human lung (Yokomizo *et al*., 2000).

In the GPPA, the two BLT receptor antagonists, ONO-4057 and U–75302, concentration-dependently inhibited the contractions induced by LTB_4, suggesting that the contractile effects of LTB_4 in the GPPA were due to the activation of BLT_1 receptors, and similar results were obtained in the human pulmonary artery.

In line with previous studies, the cyclooxygenase inhibitor indomethacin as well as the TP receptor antagonist BAY u3405 produced a significant inhibition of the contractions induced by LTB_4 in the GPLP, suggesting that a major part of this LTB_4-induced response was mediated *via* the release of TXA_2, which is further supported by the increased bath

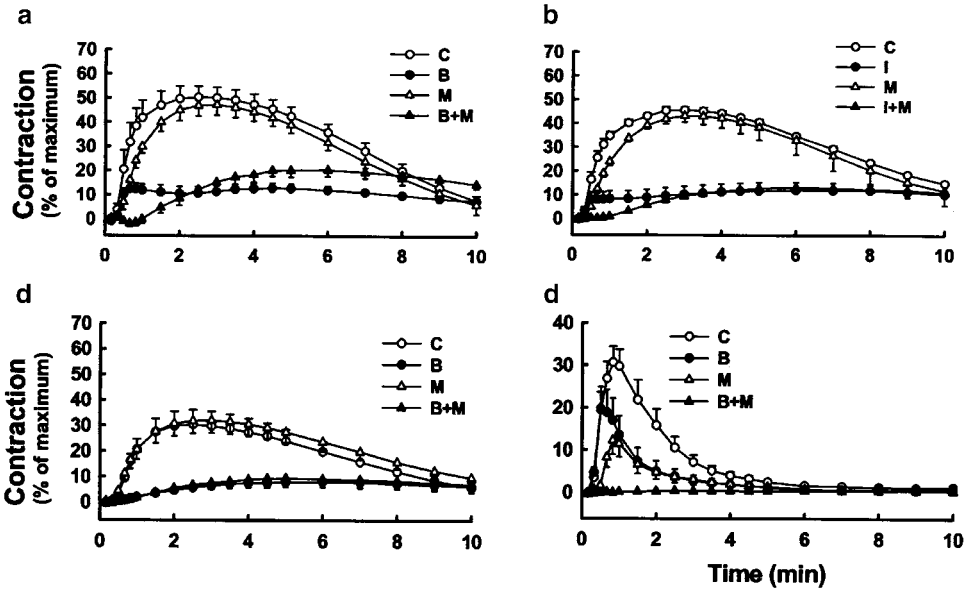

Figure 5 Effects of BAYu3405 (B), indomethacin (I) and the combination of mepyramine with metiamide (M) on the contractions induced by LTB_4 in the GPLP (100 nM; panels a–c) and pulmonary artery (1 μM; panel d). Contractions are expressed as mean $\pm$ s.e.m. of three (a, b), four (c) and seven (d) experiments.

Table 1 Effects of Indomethacin, BAYu3405, mepyramine and metiamide on the contraction induced by LTB_4 in the GPLP and GPPA

Group	n	*AUC (whole)* *0–10* min	*AUC (early)* *0–1* min
GPLP			
Control	6	325.8 $\pm$ 18.0	17.33 $\pm$ 2.42
BAY	3	104.2 $\pm$ 17.6[a]	6.84 $\pm$ 1.41[a]
Indo	3	102.9 $\pm$ 30.4[a]	5.02 $\pm$ 1.78[a]
Mep + Met	6	286.6 $\pm$ 21.6	9.23 $\pm$ 1.06[a]
BAY + Mep + Met	3	143.5 $\pm$ 21.4[a,b]	−0.71 $\pm$ 0.91[a,b]
Indo + Mep + Met	3	95.6 $\pm$ 6.8[a,b]	0.28 $\pm$ 0.12[a,b]
GPLP with U-75302 (1 μM)		0–10 min	0–1 min
Control	4	188.6 $\pm$ 36.9	6.40 $\pm$ 0.97
BAY	4	61.6 $\pm$ 15.1[a]	0.67 $\pm$ 0.38[a]
Mep + Met	4	215.9 $\pm$ 32.1	7.16 $\pm$ 1.62
BAY + Mep + Met	4	69.9 $\pm$ 24.8[a,b]	0.26 $\pm$ 0.41[a,b]
GPPA		0–5 min	0–1 min
Control	7	57.7 $\pm$ 10.9	15.83 $\pm$ 2.41
BAY	7	27.0 $\pm$ 9.4[a]	11.11 $\pm$ 3.13
Mep + Met	7	19.7 $\pm$ 6.1[a]	4.68 $\pm$ 1.31[a]
BAY + Mep + Met	7	1.2 $\pm$ 0.5[a]	−0.05 $\pm$ 0.08[a,c]

Each data represent the mean $\pm$ s.e.m. of *n* experiments. LTB_4 was administered at 100 nM (GPLP) and 1 μM (GPPA). BAY, Indo, Mep and Met means BAYu3405 3 μM, indomethacin 10 μM, mepyramine 1 μM and metiamide 1 μM, respectively.
[a] $P < 0.05$ vs control,
[b] $P < 0.05$ vs Mep + Met (Tukey test),
[c] $P < 0.05$ vs BAY or Indo.

concentrations of TXB_2 in response to LTB_4 in the GPLP. In addition, antihistamines inhibited the very early phase (0–1 min) of the LTB_4-induced contractions of the GPLP and a significant increase in histamine concentrations was detected after LTB_4 challenge. Interestingly, the histamine part of the response to LTB_4 in the GPLP was absent in the presence of the BLT_1 receptor antagonist U-75302, suggesting that the histamine component was mediated *via* BLT_1 receptor activation.

In the GPPA, the contractions induced by LTB_4 were mainly due to the liberation of histamine, as indicated by the inhibition by antihistamines of the response to LTB_4 and by measurements documenting increased concentrations of histamine in the bath fluid following exposure to LTB_4. However, also the bath concentration of TXB_2 increased after LTB_4 challenge, and addition of the TP receptor antagonist BAY u3405 abolished the residual antihistamine-resistant response to LTB_4, supporting that also TXA_2 was involved in the contractile effects of LTB_4 in the GPPA.

In summary, LTB_4 induced contractions of similar magnitude in the GPLP and GPPA, whereas only small contractions were detected in the airway preparations, suggesting that vascular components rather than airways may be the main target for LTB_4 in the lung. However, there were distinct differences between the LTB_4-induced contractions of the GPLP and GPPA. First, in the GPPA, the contractions were mediated *via* BLT_1 receptors, whereas the major part of the LTB_4 response in the GPLP was mediated *via* BLT_2 receptors. Second, although TXA_2 and histamine were released by LTB_4 in both preparations, the relative importance of the two mediators was somewhat different in the GPPA and GPLP. In addition, since the results suggest that the histamine component of both responses was mainly linked to BLT_1 receptor activation, it cannot be excluded that LTB_4 may have several different targets in the lung. For example, in addition to airways and blood vessels, the lung strip also contains other contractile elements such as alveolar ducts and interstitial cells (Kapanci *et al*., 1974). Another possibility is the release of histamine and TXA_2 from resident mast cells (Macchia *et al*., 1995), but the exact target cells for LTB_4 in the guinea-pig lung remains to be established. The failure of endothelial denudation to modify the contraction induced by LTB_4 in the GPPA suggests another effector cell in this preparation, which is

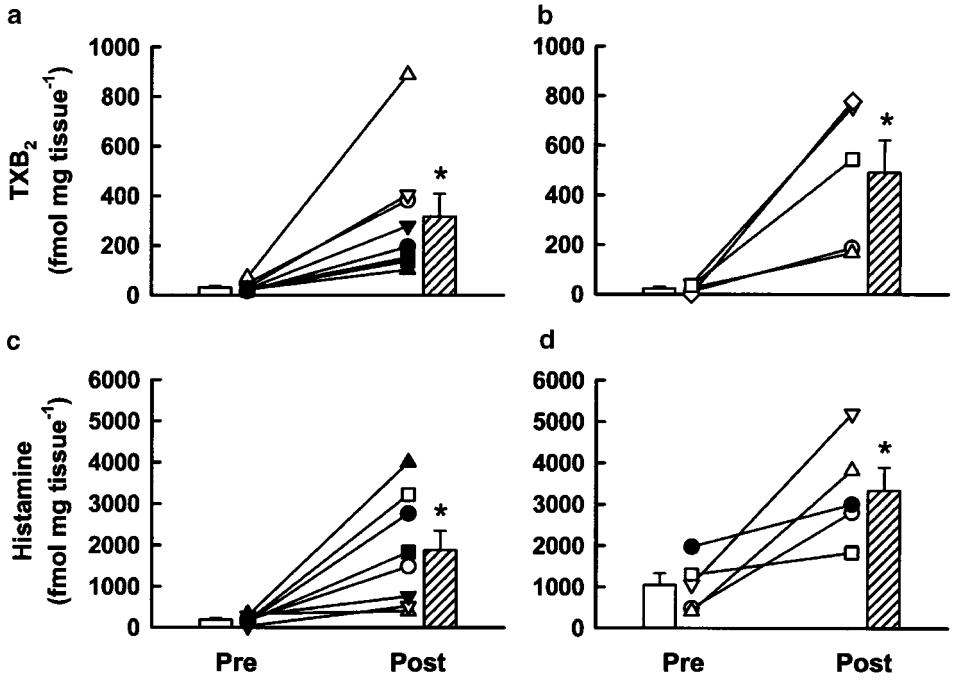

Figure 6 Measurement of thromboxane B_2 (a, b) and histamine (c, d) in the organ bath from the GPLP (a, c) and GPPA; (b, d). The bath fluid was collected either before and 15 min after LTB_4 (100 nM) treatment (GPLP) or before and 10 min after LTB_4 (1 μM) treatment (GPPA). Each point represents one experiment and bars represent mean$\pm$s.e.m. *$P<0.05$ vs pre-LTB_4 challenge (Student's *t*-test).

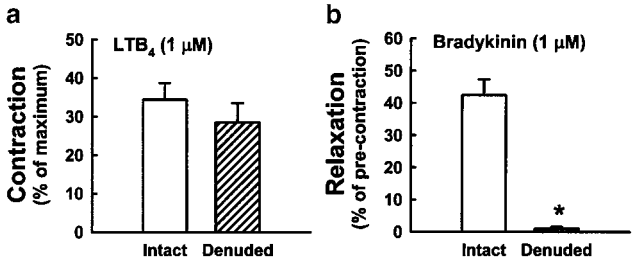

Figure 7 Effects of endothelium denudation on the contraction induced by LTB_4 (1 μM) and the relaxation induced by bradykinin (1 μM) on noradrenaline (10 μM) precontraction in the GPPA. Each bar represents the mean$\pm$s.e.m. of 9–10 experiments. *$P<0.05$ vs Intact (Student's *t*-test).

supported by previous studies that have associated vasoconstriction induced by agonists other than LTB_4 with an endothelium-independent release of either histamine (Gruetter *et al*., 1994) or TXA_2/PGH_2 (Asano *et al*., 1993; Ay *et al*., 1996).

In conclusion, the present study disclosed a new biological property of LTB_4, namely to constrict isolated pulmonary arteries. Previously, LTB_4 has primarily been regarded as a chemotactic mediator of inflammation (Ford-Hutchinson *et al*., 1980; Smith *et al*., 1980; Dahlén *et al*., 1981; Ng *et al*., 1991; Huang *et al*., 1998), and was recently also reported to be associated with T-cell recruitment (Goodarzi *et al*., 2003; Ott *et al*., 2003; Tager *et al*., 2003). The vasoconstrictive effect of LTB_4 described in the present study suggests that LTB_4 may have a wider range of biological activities and that the spasmogenic effect of LTB_4 in the lung may be an additional proinflammatory feature of this mediator.

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