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# Prostacyclin modulation of contractions of the human pulmonary artery by cysteinyl–leukotrienes

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**Abstract**

The contractile response to cysteinyl–leukotrienes was studied in isolated human pulmonary arterial rings. Concentration–response curves for leukotriene $C_4$ were significantly potentiated by the cyclooxygenase inhibitor indomethacin (1.7 μM) and after endothelial denudation. Measurements of 6-keto prostaglandin $F_{1\alpha}$ showed that cysteinyl–leukotrienes stimulated the release of prostacyclin. A single concentration (1 μM) of either leukotriene $C_4$ or leukotriene $D_4$ resulted in both contraction and relaxation. Indomethacin abolished the relaxant phase and enhanced the amplitude of the contraction, supporting that cysteinyl–leukotriene-induced contractions of the human pulmonary artery may be functionally antagonised by the release of prostacyclin. The contractions induced by leukotriene $C_4$ were resistant to the two cysteinyl–leukotriene receptor antagonists MK 571 ((3-(-2(7-chloro-2-quinolinyl)ethenyl)phenyl)((3-(dimethylamino-3-oxopropyl)thio)methyl)thio propanoic acid, 1 μM) and BAY u9773 (6(*R*)-(4′-carboxyphenylthio)-5(*S*)-hydroxy-7(*E*),9(*E*),11(*Z*)14(*Z*)-eicosatetrenoic acid, 3 μM), both in the absence and presence of indomethacin. These findings suggest a functional cysteinyl–leukotriene receptor in the human pulmonary artery with antagonist properties not previously described in human tissue.


*Keywords:* Pulmonary artery, human; Contraction; Cysteinyl–leukotriene receptor; Prostacyclin; BAY u9773; MK 571

## 1. Introduction

Cysteinyl–leukotrienes (leukotriene $C_4$, $D_4$ and $E_4$) are potent smooth muscle constrictors and have been shown to activate receptors (CysLT receptors) in the human lung (Gorenne et al., 1996). Human bronchi contain $CysLT_1$ receptors (Buckner et al., 1986; Lynch et al., 1999), and systemic $CysLT_1$ receptor antagonists are currently in clinical use for the treatment of asthma (for review, see Drazen et al., 1999). Exploring also the extrabronchial effects of cysteinyl–leukotrienes and investigating whether or not such actions are affected by the currently used anti-leukotriene drugs may contribute to our understanding of the effects and side-effects of these drugs. In animal studies, cysteinyl–leukotrienes have been shown to activate receptors on isolated pulmonary vascular preparations of different species, causing both contraction (Berkowitz et al., 1984; Ohtaka et al., 1987) and relaxation (Sakuma et al., 1987). When injected in vivo, the cysteinyl–leukotrienes increase the pulmonary arterial pressure in monkeys (Smedegård et al., 1982), rats, guinea pigs (Berkowitz et al., 1984), and pigs (Ohtaka et al., 1987).

There exist only a limited number of studies concerning the effects of cysteinyl–leukotrienes on human pulmonary vessels. Schellenberg and Foster (1984) have reported that leukotriene $C_4$ and $D_4$ but not leukotriene $E_4$ induce contractions of isolated human pulmonary arteries. The latter report together with another study (Bourdillat et al., 1987) have also concluded that the contractions induced by the cysteinyl–leukotrienes are greater in isolated human pulmonary veins compared with pulmonary arteries. These observations led to the suggestion that the arterial CysLT receptor may be different from the venous (Schellenberg and Foster, 1984). Labat et al. (1992) showed that the CysLT receptor associated with contraction of the human

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pulmonary venous smooth muscle is different from that found in human airways and this receptor is referred to as $CysLT_2$ (Coleman et al., 1995). In another study (Ortiz et al., 1995), the presence of $CysLT_1$ and $CysLT_2$ receptors on the endothelium of human pulmonary veins was also demonstrated. Activation of those endothelial receptors is associated with the release of both contractile and relaxant factors.

The immunoglobulin E (IgE)-dependent relaxations of human pulmonary arteries can mainly be explained by release of prostacyclin which is, at least in part, mediated by histamine (Ortiz et al., 1993). In addition to histamine, cysteinyl–leukotrienes are released from the human lung during antigen challenge (Dahlén et al., 1983). Since leukotriene $D_4$ previously has been described to relax isolated human pulmonary arteries (Ortiz et al., 1995), the question raised was whether or not leukotriene $C_4$ and leukotriene $D_4$ induced prostacyclin release from the human pulmonary artery. Cysteinyl–leukotrienes have previously been shown to promote prostacyclin release in cultured human umbilical vein endothelial cells (Cramer et al., 1983) as well as in isolated porcine pulmonary arteries (Bäck et al., 2000).

There are no studies that have identified which CysLT receptors are present in the human pulmonary artery. In the present report, the contractions of isolated human pulmonary arteries induced by leukotriene $C_4$ in the absence and presence of CysLT receptor antagonists were studied. The aim was to establish if the CysLT receptor in the human pulmonary artery was similar to that reported for human pulmonary venous muscle, that is, resistant to $CysLT_1$ receptor antagonism but blocked by the only known $CysLT_2$ receptor antagonist BAY u9773 (Labat et al., 1992).

## 2. Materials and methods

### 2.1. Tissue preparation

Lung tissue was obtained from 15 male and four female patients (mean age $58.6 \pm 1.7$ years) undergoing surgery for lung carcinoma. Intrapulmonary arteries were immediately dissected free from surrounding tissue and cut into rings with a length of 5 mm and an inner diameter of approximately 2–4 mm. The rings were then set up in 10 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) and gassed with 5% $CO_2$ in $O_2$ at 37°C. In some rings, the endothelium was mechanically removed by gently rubbing the luminal surface with a metal forceps.

### 2.2. Contractions

The preparations were placed under an initial resting tension of 1.5 g and changes in isometric tension were recorded using Narco F-60 force-displacement transducers connected to a Linseis 2016 polygraph. After an equilibration period of 90 min with changes of Tyrode's solution every 10 min, the preparations were incubated for 30 min in the absence (control) or presence of MK 571 (1 μM, $CysLT_1$ receptor antagonist), BAY u9773 (3 μM, $CysLT_1$ and $CysLT_2$ receptor antagonist) or indomethacin (1.7 μM, cyclooxygenase inhibitor). At the end of the 30 min treatment, a cumulative concentration effect relation for leukotriene $C_4$ was produced by the addition of increasing concentrations (1 nM–1 μM). In another series of experiments, the preparations were incubated in the absence (control) or presence of MK 571 (1 μM), BAY u9773 (3 μM), indomethacin (1.7 μM) or the combination of indomethacin and $N^{\omega}$-nitro-L-arginine (100 μM; L-NOARG, nitric oxide synthase inhibitor) for 30 min before a single concentration of either leukotriene $C_4$ or leukotriene $D_4$ (1 μM) was administered. At the end of both protocols, noradrenaline (10 μM) was administered at the plateau of the cysteinyl–leukotriene contraction, followed by acetylcholine (10 μM). The relaxation to acetylcholine indicated an intact functional endothelium, and was used as inclusion criteria for intact preparations. Likewise, the lack of relaxation to acetylcholine was used to confirm endothelial denudation.

### 2.3. Mediator release measurements

500 μl aliquots were withdrawn from the organ baths and immediately frozen at −20°C. The first sample was taken at the end of the 30 min incubation period (basal release). The following samples were taken at 2 and 20 min after administration of the single dose of cysteinyl–leukotrienes (1 μM). Direct quantification of the stable prostacyclin metabolite 6-keto prostaglandin $F_{1\alpha}$ was performed by enzyme immuno assay (Stallergenes, Fresnes, France) as described in detail by Pradelles et al. (1985).

### 2.4. Drugs

Noradrenaline, acetylcholine and indomethacin were obtained from Sigma (St. Louis, MO, USA). Leukotriene $C_4$ and $D_4$ were from Cayman Chemicals (Ann Arbor, MI, USA). BAY u9773 (6(*R*)-(4′-carboxyphenylthio)-5(*S*)-hydroxy-7(*E*),9(*E*),11(*Z*)14(*Z*)-eicosatetrenoic acid) and MK 571 ((3-(-2(7-chloro-2-quinolinyl)ethenyl)phenyl)((3-(dimethylamino-3-oxopropyl)thio)methyl)thio propanoic acid) were gifts from BAYER (Stoke Poges, Great Britain).

Solutions of cysteinyl–leukotrienes and BAY u9773 were obtained by diluting stock solutions at concentrations of 1 mM (leukotriene $C_4$), 0.5 mM (leukotriene $D_4$) and 10 mM (BAY u9773) in Tyrode's solution. Indomethacin and L-NOARG were dissolved in 1% ethanol in Tyrode's solution. Noradrenaline, acetylcholine and MK 571 were dissolved in Tyrode's solution.

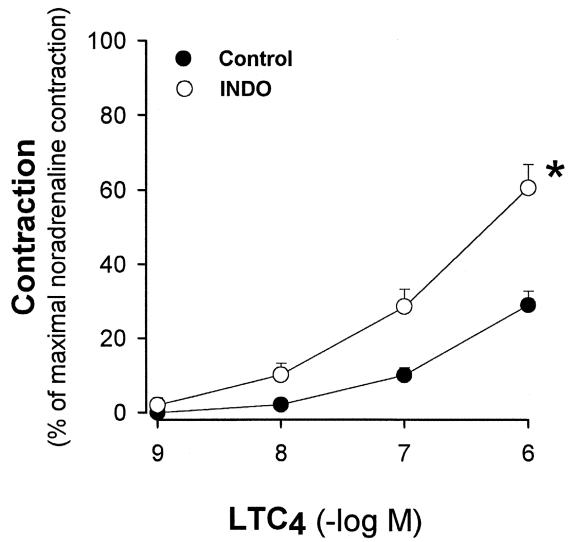


Fig. 1. Effect of the cyclooxygenase inhibitor indomethacin (INDO, 1.7 μM) on the concentration effect relation for leukotriene $C_4$ ($LTC_4$). Contractions are presented as percent of a maximal contraction to noradrenaline (10 μM) at the end of the experiment. Each point is the mean of 9–10 different lungs and vertical lines represent S.E.M. * indicates a significant difference ($P < 0.05$, two way ANOVA test) from leukotriene $C_4$ control curve.

### 2.5. Data analysis

All data are expressed as means ± S.E.M. where $n$ is the number of lung samples. The contractions are expressed as increased tension in g or as percent of the final noradrenaline response. The results of the 6-keto prostaglandin $F_{1\alpha}$ measurements are expressed as released substance in pg $min^{-1}$ $mg^{-1}$ of tissue wet weight. The net release after stimulation with cysteinyl–leukotrienes was calculated after subtracting the quantities of 6-keto prostaglandin $F_{1\alpha}$ detected before stimulation from the amounts measured post challenge.

Statistical evaluation was performed using a two way analysis of variances (ANOVA) test (concentration effect relations) or a Student's *t*-test. A *P*-value of less than 0.05 was considered significant.

## 3. Results

### 3.1. Contraction

The contractions induced by leukotriene $C_4$ (1 μM) in intact preparations were 0.42 ± 0.08 g ($n = 15$) which was not significantly different from the contractions to leukotriene $D_4$ (1 μM): 0.34 ± 0.07 g ($n = 8$). The contractions to noradrenaline (10 μM) in intact non-treated preparations were 1.7 ± 0.15 g ($n = 18$). Endothelium denudation or treatment with indomethacin and/or the CysLT receptor antagonists did not significantly alter the noradrenaline contractions. The concentration dependent contractions to cumulatively administered leukotriene $C_4$ were significantly potentiated after pretreatment with indomethacin (Fig. 1). The contractions to leukotriene $C_4$ (1 μM) were 1.1 ± 0.24 g ($n = 10$) after indomethacin treatment ($P < 0.05$ compared with untreated).

The CysLT receptor antagonists MK 571 (1 μM, $CysLT_1$ receptor antagonists) and BAY u9773 (3 μM, $CysLT_1/CysLT_2$ receptor antagonist) did not significantly alter the concentration effect relations for leukotriene $C_4$, neither in the presence nor in the absence of indomethacin (Fig. 2).

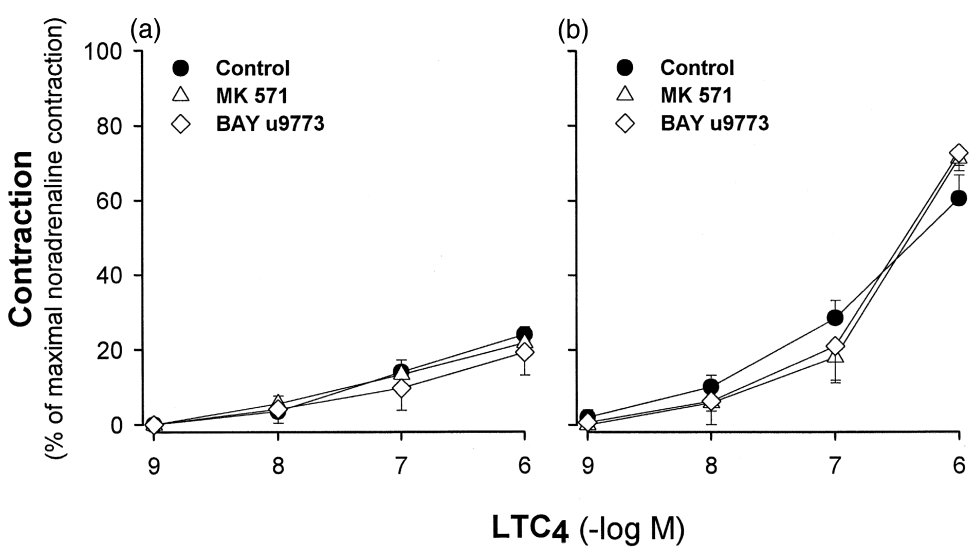


Fig. 2. Concentration effect relations for leukotriene $C_4$ ($LTC_4$) in endothelium intact human pulmonary arterial preparations in the (a) absence or (b) presence of indomethacin (1.7 μM). The preparations were treated for 30 min with the cysteinyl leukotriene receptor antagonists MK 571 (1 μM, $CysLT_1$ receptor antagonist) and BAY u9773 (3 μM, $CysLT_1/CysLT_2$ receptor antagonist). Contractions are presented as percent of a maximal contraction to noradrenaline (10 μM) at the end of the experiment. Each point is the mean of preparations derived from 4–6 different lung samples and vertical lines represent S.E.M.

In preparations where the endothelium had been rubbed away, the concentration–response curve to leukotriene $C_4$ was potentiated compared with controls (Fig. 3, for controls see Fig. 1). In addition, in the rubbed preparations the concentration–response curves for leukotriene $C_4$ and leukotriene $D_4$ were similar (Fig. 3). BAY u9773 (3 μM) did not significantly alter the contractions to leukotriene $C_4$ in rubbed preparations (Fig. 3).

When administered as a single dose (final bath concentration: 1 μM) both leukotriene $C_4$ ($n = 5$) and leukotriene $D_4$ ($n = 5$) induced a triphasic response, starting with an initial contraction of $16 \pm 4.1\%$ and $13 \pm 3.2\%$, respectively. This contraction rapidly turned into a relaxation with its maximum after 2 min. This was followed by a slow contraction, reaching its plateau after 20 min. For leukotriene $C_4$ the plateau was $18 \pm 4.9\%$ and for leukotriene $D_4$ $18 \pm 6.5\%$. A representative tracing of the triphasic response is shown in Fig. 4. MK 571 (1 μM) and BAY u9773 (3 μM) did not prevent the triphasic response to leukotriene $C_4$ ($n = 2$ for each antagonist, not shown). Indomethacin pretreatment abolished the relaxant component and enhanced the amplitude for both leukotriene $C_4$ ($n = 4$, Fig. 4) and leukotriene $D_4$ ($n = 1$, not shown). After indomethacin pretreatment, the contractions at the plateau were $46 \pm 1.6\%$ for leukotriene $C_4$ ($n = 4$, $P < 0.05$ compared with controls). The combination of indomethacin and the nitric oxide synthesis inhibitor L-NOARG did not further potentiate the response to leukotriene $C_4$ compared with preparations treated with indomethacin alone ($n = 2$, not shown).

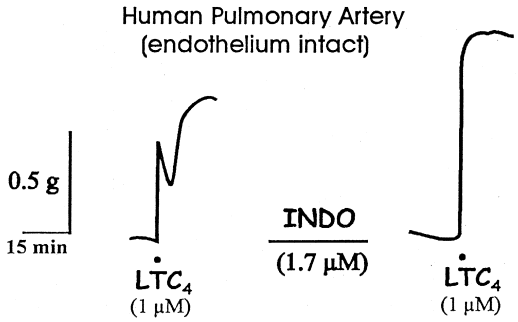


Fig. 4. Tracings from organ bath experiments with endothelium intact human pulmonary arterial ring preparations. The left panel shows the triphasic response to leukotriene $C_4$ ($LTC_4$; 1 μM) under control conditions. After 30 min treatment with the cyclooxygenase inhibitor indomethacin (INDO; 1.7 μM) the contraction was monophasic with enhanced amplitude.

### 3.2. Mediator release measurements

The release of prostacyclin (determined by measurements of 6-keto prostaglandin $F_{1\alpha}$) into the organ bath fluid of the isolated human pulmonary arterial preparations was significantly increased after stimulation with either leukotriene $C_4$ or leukotriene $D_4$ (Fig. 5). The release was most prominent 2 min after cysteinyl–leukotriene administration, and there were no significant differences between the release caused by either leukotriene $C_4$ or leukotriene $D_4$ at the times studied.

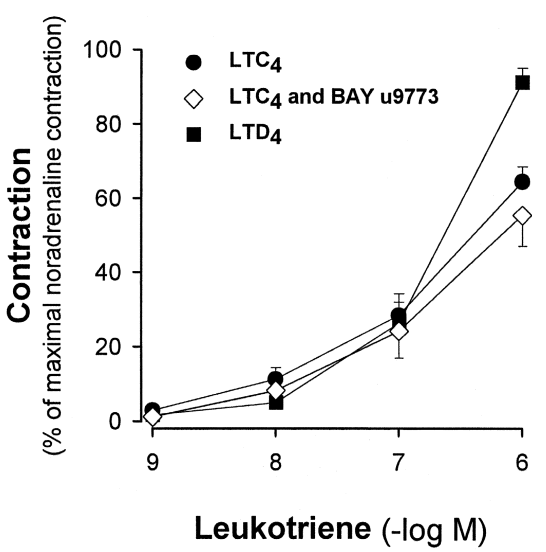


Fig. 3. Concentration effect relations for leukotriene $C_4$ ($LTC_4$) control, leukotriene $C_4$ after pretreatment with BAY u9773 (3 μM) and leukotriene $D_4$ ($LTD_4$) control, in rubbed human pulmonary arterial preparations. Contractions are presented as percent of a maximal contraction to noradrenaline (10 μM) at the end of the experiment. Each point is the mean of preparations derived from 4–6 different lung samples and vertical lines represent S.E.M.

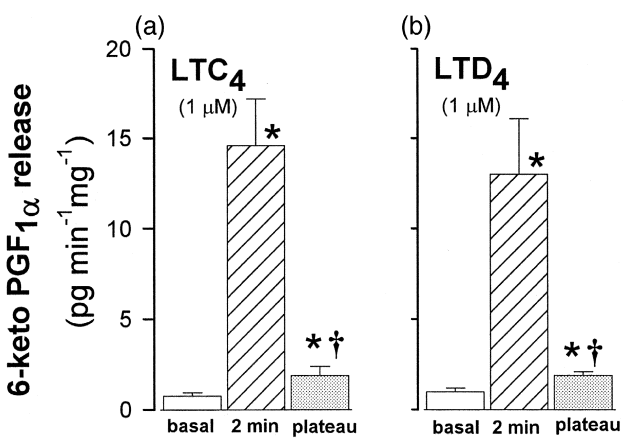


Fig. 5. Measurements of the stable prostacyclin metabolite 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) after stimulation with either (a) leukotriene $C_4$ ($LTC_4$; 1 μM) or (b) leukotriene $D_4$ ($LTD_4$; 1 μM). Each bar represents the mean ± S.E.M. of samples from five (leukotriene $C_4$) or four (leukotriene $D_4$) different lungs. * indicates a significant difference ($P < 0.05$, Student's *t*-test) compared with basal release and † indicates a significant difference compared with the 2-min value. There were no significant differences between leukotriene $C_4$ and leukotriene $D_4$ at the times studied. The increased release at 2 min corresponded to the relaxant phase of the functional response to both agonists.

## 4. Discussion

In isolated human pulmonary arteries, the concentration–response curves for leukotriene $C_4$ were significantly potentiated by endothelial denudation as well as after cyclooxygenase inhibition. A single concentration of either leukotriene $C_4$ or leukotriene $D_4$ (1 μM) resulted in both contraction and relaxation. Indomethacin abolished the relaxant phase of this response and enhanced the maximal amplitude. In addition, there was a significant amount of 6-keto prostaglandin $F_{1\alpha}$ released during the cysteinyl–leukotriene-induced contractions. The $CysLT_1$ receptor antagonist MK 571, as well as the dual $CysLT_1/CysLT_2$ receptor antagonist BAY u9773 failed to significantly modify the contractions to leukotriene $C_4$, both in the absence and presence of indomethacin.

The contractions to leukotriene $C_4$ and $D_4$ in isolated human pulmonary arteries were relatively small when compared with the response to noradrenaline or to previously published data obtained in human bronchial and pulmonary venous preparations (Buckner et al., 1986; Labat et al., 1992). These results (present report) corroborate with the initial studies in human pulmonary arteries (Schellenberg and Foster, 1984; Bourdillat et al., 1987) and may explain why so few studies on cysteinyl–leukotriene responses have been performed in this tissue. The present study indicates that this relatively weak contractile response may be partially explained as modulation of the cysteinyl–leukotriene contractions by factors of the cyclooxygenase pathway, since the contractions to leukotriene $C_4$ were significantly potentiated by indomethacin. After indomethacin pre-treatment, the contractions of the preparations were similar to what have previously been reported for cysteinyl–leukotrienes in isolated human bronchi and pulmonary veins (Schellenberg and Foster, 1984; Buckner et al., 1986; Bourdillat et al., 1987; Labat et al., 1992). In addition, an identical potentiation was observed when the endothelium was removed (rubbed), suggesting that leukotriene $C_4$ stimulates the endothelium to release relaxant cyclooxygenase products that under normal conditions act upon the vascular smooth muscle to attenuate the contraction. When this counteracting mechanism had been removed, the contractions to cysteinyl–leukotrienes in the human pulmonary artery were similar to what has been observed in other preparations, for example isolated porcine pulmonary arteries (Ohtaka et al., 1987; Bäck et al., 2000).

There are no previous reports concerning the inhibitory effects of CysLT receptor antagonists on the contraction of human pulmonary arteries. The data (present report) demonstrate that the $CysLT_1$ receptor antagonist MK 571 did not block the leukotriene $C_4$ contraction in isolated human pulmonary arteries. This antagonist has previously been shown to be very potent and selective at the $CysLT_1$ receptor (Jones et al., 1989). According to the current IUPHAR definition of CysLT receptors, the receptor responsible for a functional response resistant to $CysLT_1$ receptor antagonists is named $CysLT_2$ (Coleman et al., 1995). The leukotriene $E_4$ analogue BAY u9773 has been shown to inhibit contractions that are resistant to $CysLT_1$ receptor antagonists, in the human pulmonary vein (Labat et al., 1992), guinea pig trachea (Tudhope et al., 1994), guinea pig ileum (Bäck et al., 1996) and sheep trachea (Wikström Jonsson, 1997). In the human pulmonary artery (present report), the contractions to leukotriene $C_4$ were resistant to also this antagonist, suggesting the presence of another CysLT receptor subtype in human pulmonary arteries. The resistance of the leukotriene $C_4$ contractions to $CysLT_1$ and $CysLT_2$ receptor antagonism was present in endothelium intact preparations, both in the absence and presence of indomethacin. In addition, the same pattern was observed in preparations where the endothelium had been removed. The latter observation is identical to the findings in the porcine pulmonary artery (Bäck et al., 2000), suggesting that the porcine preparation may be used as a relevant model for the human pulmonary artery.

The measurements of 6-keto prostaglandin $F_{1\alpha}$ (Fig. 5) showed that both leukotriene $C_4$ and leukotriene $D_4$ stimulated the release of prostacyclin in the human pulmonary artery. This release was most prominent during the first 2 min after stimulation. The functional response to the same treatment was triphasic, with an initial rapid contraction followed by a relaxation and then a slow contraction (Fig. 4). The relaxant phase of this response was maximal 2 min subsequent to cysteinyl–leukotriene stimulation, which corresponded to the measured maximal increase in prostacyclin release. This suggests that prostacyclin may be responsible for the relaxant component of the triphasic response to cysteinyl–leukotrienes. This suggestion is further supported by the finding (present report) that indomethacin treatment not only enhanced the amplitude of the contraction, but also abolished the relaxant phase. In spiral strips of human pulmonary artery, prostacyclin release has previously been suggested as the explanation of a similar triphasic response to histamine (Schellenberg et al., 1986). In addition, a recent report (Walch et al., 1999) demonstrated that only the IP receptor is involved in the prostanoid induced relaxations of the human pulmonary artery.

Cramer et al. (1983) previously reported that the release of prostacyclin induced by cysteinyl–leukotrienes in cultured human umbilical vein endothelial cells was resistant to the nonselective CysLT receptor antagonist FPL 55712. Likewise, in porcine pulmonary arterial preparations, selective $CysLT_1$ receptor antagonism as well as the dual $CysLT_1/CysLT_2$ receptor antagonist BAY u9773 did not prevent the release of 6-keto prostaglandin $F_{1\alpha}$ induced by either leukotriene $C_4$ or leukotriene $D_4$ (Bäck et al., 2000). In the present study, the prostacyclin modulation of the responses to leukotriene $C_4$ were observed also in the presence of the CysLT receptor antagonists MK 571 and BAY u9773, suggesting that the receptor responsible for the cysteinyl–leukotriene-induced prostacyclin release was resistant to those antagonists. Ortiz et al. (1995) previously

reported that $CysLT_1$ receptor antagonists did not affect endothelium-dependent relaxations to leukotriene $D_4$ in the human pulmonary artery.

The contractions to noradrenaline were not altered either by treatment with indomethacin or when the endothelium was rubbed away (present report; Ortiz et al., 1993; Martínez et al., 1995). This supports the notion that the release of prostacyclin in response to leukotriene $C_4$ and $D_4$ was receptor mediated rather than a general response to muscle contraction. Ortiz et al. (1992) showed that both prostacyclin and nitric oxide are involved in regulating functional responses to histamine in the human pulmonary artery. In addition, the leukotriene $D_4$ responses in the human pulmonary veins are significantly potentiated after nitric oxide synthesis inhibition (Ortiz et al. 1995). In the present report, a combination of indomethacin and the nitric oxide synthesis inhibitor L-NOARG did not further potentiate the contractile responses to leukotriene $C_4$ compared with indomethacin alone. This suggests that nitric oxide may play a minor modulatory role in the leukotriene $C_4$ induced responses of intact human pulmonary arteries. This is further supported by the observation (present report) that endothelium denudation produced a potentiation of leukotriene $C_4$ responses which was identical to that observed after indomethacin treatment.

In conclusion, the present study provides evidence that the leukotriene $C_4$ induced contractions of human pulmonary arteries are mediated via a class of CysLT receptors resistant to $CysLT_1$ receptor antagonism, suggesting that the clinically used anti-asthmatic $CysLT_1$ receptor antagonists may not affect vasoregulation in the pulmonary arterial vasculature. Interestingly, the human pulmonary arterial receptor exhibited a different profile compared with the receptor present in human pulmonary venous smooth muscle, where the contractions to cysteinyl–leukotrienes are resistant to $CysLT_1$ receptor antagonism but inhibited by BAY u9773 (Labat et al., 1992). In addition, the contractions of the human pulmonary artery were functionally antagonised by the release of prostacyclin from the endothelium. Neither $CysLT_1$ nor $CysLT_2$ receptor antagonism reversed this functional antagonism, suggesting that the same receptor is mediating both contraction and prostacyclin release.

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